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(54) Hybridization probes derived from the spacer region between the 16S and 23S rRNA genes for the detection of non-viral microorganisms.

(57) The invention relates to a probe consisting of at least about 15 oligonucleotides of the spacer region between rRNA genes of a non viral organism, particularly prokaryotic organism and more particularly bacteria, and preferably from about 15 oligonucleotides to about the maximum number of oligonucleotides of the spacer region and more preferably from about 15 to about 100 oligonucleotides.

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The invention relates to nucleic acid probes derived from the spacer region between the ribosomal ribonucleic acid (rRNA) gene, particularly between the 16S and 23S rRNA genes, to be used for the specific detection of non-viral organisms in a biological sample by a hybridization procedure.

Although much progress has been made in the last decade, for many microorganisms the diagnostic procedures currently in use are still laborious, nonsensitive and aspecific. Many of these pitfalls can be overcome by using nucleic acid probes. These nucleic acid probes can, for instance be total genomic deoxyribonucleic acid (DNA), plasmids, riboprobes or synthetic oligonucleotides and these probes may target the genomic DNA or messenger or stable RNA species present in biological samples. Although not necessary, the use of synthetic oligonucleotides is preferred. Oligonucleotides can be rapidly synthesized in great amount, using chemical methods, have a long shelf-life, and are easily purified and labeled.

For a reliable diagnosis of microorganisms using the DNA-probe technology the probes used should be highly specific (i.e. they should not cross-react with nucleic acids from other organisms) and highly sensitive (i.e. most, if not all, strains of the organism to be detected should react with the probe). Hence, the preferred target sequences should have the following characteristics :

(i) The sequence should be present in the genome of each strain of the organism concerned.

(ii) The evolutionary diversity of the sequence should be such that on one hand there is sufficient sequence-diversity to allow differentiation of the species concerned from other closely related species, and on the other hand, sufficient sequence-conservation to allow the detection of all strains of the species concerned with the probe used.

Species-specific probes have been described for a great number of organisms. For a recent review see Tenover, Clin. Microbiol. Rev. 1 : 82-101, 1988.

However, it is not obvious from which gene in the genome specific probe sequences can be derived. In probe development often large selection procedures have to be followed to obtain fragments which at last turn out to be specific for the organism under investigation (KOROLIK et al., J. Gen. Microbiol. 134: 521-529, 1988; GRIMONT et al., J. Clin. Microbiol. 21: 431-437, 1985; WELCHER et al., Nucl. Acids Res. 14: 10027-10044, 1986; DONEGAN et al., Mol. Cell. Probes 3 : 13-26, 1989; BEAULIEU and ROY, Abstract nr D249, Abstracts of the Annual Meeting of the American Society for Microbiology, 1989). Most often the function or identity of the gene from which the specific fragment derives is not known and the screening procedure has to be blindly repeated each time another specific probe is wanted. The precise identification of a gene which meets the criteria listed above and which is ubiquitous present would obviate the need for time consuming and tedious selections.

The 16S or 23S rRNA genes are quite often used for probe development since sequences can easily be obtained using described methods and it is known that variable regions exist within these highly conserved genes which can be used for species specific detection. However, for certain organisms it may not be possible to derive highly specific and sensitive probes from the 16S and 23S rRNA genes for instance because their evolutionary nucleic acid sequence conservation is too high. Another consequence of the conserved character of these genes is that the differentiation of two organisms is often based on one or a few mismatches only in the target sequence which puts constraints on the stringency of the hybridization. A slight deviation from these conditions may result in misidentification.

Therefore the characterization of a ubiquitous gene which allow the development of species specific probes for most organisms including those for which it was not possible to infer specific from the 16S and 23S rRNA genes, and which preferably have a broader stringency-range, would be extremely advantageous.

Each cellular organism possesses ribosomal RNA cistrons since its transcripts are essential for the function of ribosomes and the synthesis of proteins. In general the genes are present in multiple copies in the genome. In eubacteria the 16S rRNA gene [also called small subunit rRNA (srRNA)] is found at the 5' end of the rRNA cistron, followed by the 23S rRNA [also called large subunit rRNA(lrRNA)]. The 5S rRNA gene is located at the 3' end of the cistron. The 16S, 23S and 5S genes are separated by spacer regions in which transfer RNA (tRNA) genes and signal sequences involved in post-transcriptional processing may be found. At first the rRNA cistron is transcribed as one precursor RNA molecule. This primary transcript is further processed by endo- and exoribonucleases to its mature products. As a consequence, spacer region sequences are not exclusively present in the genome of the organism but also in precursor RNA molecules and processing products. The structure and processing of eubacterial rRNA cistrons is discussed in detail in the following reference : GEGENHEIMER and APIRION, Microbiol. Rev. 45 : 502-541, 1981.

The situation in nuclear genomes of eukaryotes somewhat differs in that a 5.8S RNA gene is located between the srRNA and lrRNA and 5S rRNA genes are arranged in separate long tandem arrays (PERRY, Annu. Rev. Biochem. 45 : 605-629, 1976; LONG and DAWID, Annu. Rev. Biochem. 49 : 727-764, 1980.). However, rRNA cistrons in the mitochondria or chloroplasts of eukaryotic organisms are prokaryotic in nature (BORST and GRIVELL, Nature 290 : 443-444, 1981).

The nucleic acid sequence of the spacer region of only a very limited number of eukaryotic or prokaryotic organisms is available from the literature (e.g. YOUNG et al., J. Biol. Chem. 254 : 3264-3271, 1979; and MARTENS et al., System. Appl. Microbiol. 9: 224-230, 1987.). From these data no reliable estimation of the nucleic acid sequence conservation can be made and consequently nothing can be concluded concerning the suitability of the spacer region for the selection of specific probes.

More precisely, concerning prokaryotes, hybridization probes derived from the spacer region between the 16S and 23S rRNA genes for the detection of microorganisms in a biological sample have not yet been described. Neither it is known for the corresponding spacer region between the small and large subunit rRNA genes of eukaryotes.

As far as eukaryotes are concerned, the use of a cloned fragment from a ribosomal gene spacer has been described in a taxonomical study on Leishmania (Ramirez and Guevara, Mol. Bioch. Parasitol. 22: 177-183, 1987). However, the region used as well as as the approach of the study are no teaching, for the man skilled in the art, to use a probe derived from the spacer region between the small rRNA and large rRNA genes, particularly for the following reasons :

(i) the ribosomal genes spacer used by Ramirez and Guevara, is not the spacer region between the srRNA and lrRNA, but refers to the sequence present between two adjacent rRNA cistrons; such spacers are only found in eukaryotes between repeating units of rRNA cistrons and are not related to the internal spacer in between the srRNA and lrRNA genes;

(ii) the differentiation between Leishmania taxa using the gene spacer fragment is achieved by comparing restriction fragment patterns;

the fragment used is not specific; hence, differentiation with the fragment using a simple hybridization protocol without resorting to Southern-Blot analysis is not possible.

No evidence is presented that highly specific probes can be found in that ribosomal gene spacer.

Thus, the aim of the invention is to provide species specific probes derived from the spacer region between rRNA genes for a particular organism such as a bacterial species.

Another object of the invention is to provide for DNA probes derived from the 16S-23S rRNA spacer region for the detection of Neisseria gonorrhoeae, Neisseria meningitidis, Branhamella catarrhalis, Haemophilus ducreyi and Bordetella pertussis strains.

Still, another object of the invention is to provide DNA probes derived from the 16S-23S rRNA gene spacer region for the detection of Neisseria gonorrhoeae, Neisseria meningitidis, Branhamella catarrhalis, Haemophilus ducreyi and Bordetella pertussis strains in a biological sample by a hybridization test such as a dot-spot, a strand-displacement, a competition, or a sandwich hybridization test.

Still another object of the invention is to provide probes and a simple method for the in vitro diagnosis of Neisseria gonorrhoeae, Neisseria meningitidis, Branhamella catarrhalis, Haemophilus ducreyi and Bordetella pertussis strains.

The invention relates to a probe consisting of at least about 15 oligonucleotides of the spacer region between rRNA genes of a non viral organism, particularly prokaryotic organism and more particularly bacteria.

The invention relates more particularly to a probe consisting from about 15 oligonucleotides to about the maximum number of oligonucleotides of the spacer region, and more preferably from about 15 to about 100 oligonucleotides of the spacer region between the rRNA genes, particularly between 16S and 23S rRNA genes of a non viral organism, particularly prokaryotic organisms, and more particularly bacteria.

In the following, the expression "spacer region" designates the spacer region between rRNA genes and more particularly between the 16S and 23S rRNA genes.

The invention relates to a probe for use in a hybridization assay, liable to be obtained in the process which comprises constructing an oligonucleotide that is sufficiently complementary to hybridize to a sequence of the spacer region between rRNA genes selected to be unique to non-viral organisms, particularly prokaryotic organisms, more particularly bacteria, sought to be detected, said sequence of the spacer region between rRNA genes being selected

- either by
  - comparing the nucleotide sequence of the spacer region between the rRNA genes of the sought organism with the nucleotide sequence of the spacer region between the rRNA genes of the closest neighbours,
  - selecting a sequence of about at least 15 oligonucleotides, and preferably from about 15 to about the maximum number of oligonucleotides of the spacer region and more preferably from about 15 to about 100 oligonucleotides, of the spacer region between rRNA genes of the sought organism which presents at least one mismatch with the spacer region between the rRNA genes of at least one of the closest neighbours,

- or by
  * deleting, in the spacer region of the organism to be sought, the tRNA genes and possibly the signal sequences, to obtain a shortened spacer region and
  * determining by trial and error a specific nucleotide sequence of at least about 15 oligonucleotides, and preferably from about 15 to about the maximum number of oligonucleotides of the spacer region and more preferably from about 15 to about 100 oligonucleotides, from the shortened spacer region, said sequence being able to hybridize specifically with the nucleic acids (DNA and/or RNAs) of the sought organism.

The invention relates particularly to a probe wherein the spacer region between rRNA genes is the spacer region between the 16S rRNA gene and the 23S rRNA gene.

The spacer regions of several microorganisms were cloned, sequenced and compared as will be outlined later herein. The comparison revealed that the nucleic acid sequence of the spacer region is of a semi conserved nature, as compared to rRNA genes, which are highly conserved. Hence the spacer region might be better suited for probe development than the rRNA genes itself. The figures 1 and 2 illustrate that there is a high degree of sequence homology between highly related organisms (such as highly related strains from the same genospecies). Somewhat related organisms as shown in figure 3 and 7. A total lack of significant sequence homology (except for the tRNA sequences) could be demonstrated between distantly related species, as shown in figures 4 to 6.

In the table below, homology values (in % sequence homology) of 16S rRNA sequences of different strains (16S hom) are compared with the corresponding homology values of the spacer regions (spacer hom). The homology values (16S hom and spacer hom.) were calculated using the PC gene software supplied by Intelligentics Inc. and Genofit SA (release 6.01/April 20, 1989). Between brackets the total number of nucleotides compared is given. The results clearly show that the spacer region is less conserved than the 16S rRNA molecule.

strains compared			
strain 1	Strain 2	16S hom	Spacer hom
N. gonorrhoeae NCTC 8375	N. gonorrhoeae ITG 4367	99.9% (1434)	100% (335)
B. pertussis ATCC 10380	B. bronchiseptica NCTC 452	100% (417)	98.1% (582)
N. gonorrhoeae NCTC 8375	N. meningitidis NCTC 10025	99% (1452)	93.5% (603)
B. catarrhalis ITG 4197	M. nonliquefaciens ATCC 19975	97.9% (1244)	87.1% (498)
B. pertussis ATCC 10380	N. gonorrhoeae NCTC 8375	86.3% (998)	58.4% (582)
B. catarrhalis ITG 4197	N. gonorrhoeae NCTC 8375	83.8% (1485)	68.1% (498)
H. ducreyi CIP 541	E. coli	88.3% (1498)	67.1% (346)

As a result, highly species-specific and sensitive probes could be inferred from the spacer region sequence of the relevant pathogenic species under study i.e. Neisseria gonorrhoeae, Neisseria meningitidis, Branhamella catarrhalis, Haemophilus ducreyi and Bordetella pertussis. It was even so that for the species Neisseria meningitidis and Bordetella pertussis for which highly specific probes could not be found in the 16S and/or 23S rRNA molecules, valuable probes could be derived from the spacer region.

It is very likely that specific probes for other species than those described herein (e.g. Campylobacter species, other Haemophilus species, etc. ...) can be inferred from spacer region sequences as well.

The target of the probes derived from the spacer region between the 16S and 23S rRNA gene is the genomic DNA and the precursor RNA molecules present in the cells to be detected. The detection of the precursor RNA molecules is advantageous since these molecules are single stranded and may be present in multiple copies. On the other hand DNA molecules are much more refractory to enzymatical degradation than RNA molecules. Hence DNA targeting is preferred when biological samples cannot be processed and/or stored adequately to prevent RNA degradation prior to hybridization.

Another particular advantage of probes derived from the 16S - 23S rRNA spacer regions lies in target detection after enzymatical amplification using the polymerase chain reaction (PCR). The spacer region of many microorganisms can for instance be enzymatically amplified using the same primers allocated in a conversed region of the 3' end and the 5' end of the 16S and the 23S rRNA genes respectively. Taking advantage of the highly conversed character of the rRNA genes, spacer regions of many organisms can be amplified, if preferred simultaneously, using the same reagents and protocol and afterwards the amplified fragment can be detected using a probe which specifically targets the spacer region of the organism of

interest.

Since the spacer region is flanked by conserved sequences the cloning and sequencing of this region with the aid of the PCR technique is simple, and the same protocol can be applied to a great variety of organisms. Hence the sequences of the spacer regions are obtained by enzymatical amplification of rRNA genes using conserved primers allocated in the 16S or 23S rRNA. Examples of basic primer-pairs which can be used for the amplification of fragments spanning the spacer region are :

Primer pair 1 : TGGCTCAGAT TGAACGCTGG CGGC, and
CCTTTCCCTC ACGGTACTGG T
Primer pair 2 : TGGGTGAAGT CGTAACAAGG TA, and
CACGTCCTTC GTCGCCT.

The amplified fragment can be cloned as such or as two sub-fragments after digestion with a restriction-enzyme recognizing a unique restriction-site. A strategy for cloning PCR products in M13 has been described by Medlin et al. (Gene 71:491-499, 1988).

The same strategy can be used for cloning in a plasmid vector. In this approach the basic primers are extended at their 5' end with a nucleotide sequence comprising an unique restriction site, enabling directional cloning of the fragment. After cloning in a plasmid vector the spacer region can be sequenced using the dideoxy chain termination method.

This approach is considerably less tedious and time-consuming than the conventional cloning procedures using genomic banks or selected restriction endonuclease fragments.

Although sequence information is more rapidly obtained when the sequencing reactions are performed directly on PCR fragments without cloning, the sequence information generated from cloned fragments is more accurate and complete. In contrast to PCR fragments, cloned gene fragments can easily be purified in great amounts, which results in clearly readable sequencing ladders. Since one mismatch in the probe-sequence may result in useless probes, accuracy is highly preferred over the speed of obtaining sequences.

Taking into account the ease of obtaining spacer sequences with the approach outlined above, nucleotide sequence comparison of the spacer region of the organism for which a probe is desired with the spacer region of the closest neighbour, is the preferred way to infer specific probe sequences.

The closest neighbour means the taxon which is known to be most closely related in terms of DNA homology and which has to be differentiated from the organism of interest.

Depending on the taxonomical position of the organism of interest, the closest neighbour may be extremely highly related to the organism of interest, exhibiting more than 75% degree of binding, or may be rather distantly related showing no significant percentage of DNA homology. In the initial renaturation rate method degree of binding values are insignificant below about 30%; in solid phase DNA:DNA hybridization methods DNA homologies become insignificant between 10 to 20% degree of binding.

However, when the nucleotide sequences of the closest neighbors from which the organism of interest has to be differentiated, are not available, the selection of the specific probes can be done by trial and error. In that case, for each particular organism a specific probe region, which may be located anywhere in the spacer region, has to be defined experimentally. Only few areas in the spacer regions, such as tRNA genes or signal sequences, can in certain instances, be excluded a priori as probe regions. However, since 16S-23S rRNA spacer regions in general are small -- usually not longer than 700 bp -- good probe sequence can be readily found without extensive screening.

By way of example, for a spacer region between the 16S and 23S rRNA gene of 700 bp, the "shortened" spacer region obtained by deleting the tRNA gene and the signal sequence can be of about 500 pb.

With a "biological sample" is meant a specimen such as a clinical sample (pus, sputum, blood, urine, etc.), a environmental sample, bacterial colonies, contaminated or pure cultures, purified nucleic acid, etc... in which the target sequence of interest is sought.

"rRNA gene spacer region derived" as used herein, refers to the fact that the probes concerned hybridize with sequences located in the spacer region between ribosomal RNA genes normally present in the genome or transcript RNA molecules, no matter whether said probes are themselves formed of DNA or RNA fragments, or whether they consist of cloned fragments (in the case of DNA) or of synthetic oligonucleotides.

A hybridization probe of the invention for detecting Neisseria gonorrhoeae strains contains :

- either a sequence belonging to a nucleic acid selected from the following group of nucleic acids and which includes itself of from 15 to the maximum number of nucleotides of the selected nucleic acid,

Group NGI1 :

CGATGCGTCG	TTATTCTACT	TCGC	NGI1
GCGAAGTAGA	ATAACGACGC	ATCG	NGI1IC
GCGAAGUAGA	AUAACGACGC	AUCG	NGI1ICR
CGAUGCGUCG	UUAUUCUACU	UCGC	NGI1R

- or a variant sequence which differs from any of the preceding sequences
  - either by addition to or removal from any of their respective extremities of one or several nucleotides,
  - or changing within any of said sequences of one or more nucleotides,
  - or both,

yet provided that in any of the above circumstances said probe still hybridizes with the same RNA or DNA target as the corresponding unmodified sequence.

A hybridization probe of the invention for detecting Neisseria meningitidis strains contains :

- either a sequence belonging to a nucleic acid selected from the following group of nucleic acids and which includes itself of from 15 to the maximum number of nucleotides of the selected nucleic acid,

Group NMI1 :

GGTCAAGTGT	GACGTCGCCC	TG	NMI1
CAGGGCGACG	TCACACTTGA	CC	NMI1IC
CAGGGCGACG	UCACACUUGA	CC	NMI1ICR
GGUCAAGUGU	GACGUCGCCC	UG	NMI1R

Group NMI2 :

GTTCTTGGTC	AAGTGTGACG	TC	NMI2
GACGTCACAC	TTGACCAAGA	AC	NMI2IC
GACGUCACAC	UUGACCAAGA	AC	NMI2ICR
GUUCUUGGUC	AAGUGUGACG	UC	NMI2R

Group NMI3 :

GCGTTCGTTA	TAGCTATCTA	CTGTGC	NMI3
GCACAGTAGA	TAGCTATAAC	GAACGC	NMI3IC
GCACAGUAGA	UAGCUAUAAC	GAACGC	NMI3ICR
GCGUUCGUUA	UAGCUAUCUA	CUGUGC	NMI3R

Group NMI4 :

TGCGTTCGAT	ATTGCTATCT	ACTGTGCA	NMI4
TGCACAGTAG	ATAGCAATAT	CGAACGCA	NMI4IC
UGCACAGUAG	AUAGCAAUAU	CGAACGCA	NMI4ICR
UGCGUUCGAU	AUUGCUAUCU	ACUGUGCA	NMI4R

Group NMI5 :

TTTTGTTCTTGGTCAAGTGTGACGTCGCCCTGAATGGATTCTGTTCCATT

NMI5

AATGGAACAGAATCCATTCAGGGCGACGTCACACTTGACCAAGAACAAAA

NMI5C

AAUGGAACAGAAUCCAUUCAGGGCGACGUCACACUUGACCAAGAACAAAA

NMI5ICR

UUUUGUUCUUGGUCAAGUGUGACGUCGCCCUGAAUGGAUUCUGUUCCAUU

NMI5R

- or a variant sequence which differs from any of the preceding sequences
  - either by addition to or removal from any of their respective extremities of one or several nucleotides,
  - or changing within any of said sequences of one or more nucleotides,
  - or both,

yet provided that in any of the above circumstances said probe still hybridizes with the same RNA or DNA target as the corresponding unmodified sequence.

A hybridization probe of the invention for detecting Branhamella catarrhalis strains contains :

- either a sequence belonging to a nucleic acid selected from the following group of nucleic acids and which includes itself of from 15 to the maximum number of nucleotides of the selected nucleic acid,

Group BCI1 :

TTAAACATCT TACCAAAG	BCI1
CTTTGGTAAG ATGTTTAA	BCI1IC
CUUUGGUAAG AUGUUUAA	BCI1ICR
UUAAACAUCU UACCAAAG	BCI1R

- or a variant sequence which differs from any of the preceding sequences
  - either by addition to or removal from any of their respective extremities of one or several nucleotides,
  - or changing within any of said sequences of one or more nucleotides,
  - or both,

yet provided that in any of the above circumstances said probe still hybridizes with the same RNA or DNA target as the corresponding unmodified sequence.

A hybridization probe of the invention for detecting Haemophilus ducreyi strains contains :

- either a sequence belonging to a nucleic acid selected from the following group of nucleic acids and which includes itself of from 15 to the maximum number of nucleotides of the selected nucleic acid,

Group HDI1 :

TTATTATGCG CGAGGCATAT TG	HDI1
CAATATGCCT CGCGCATAAT AA	HDI1IC
CAAUAUGCCU CGCGCAUAAU AA	HDI1ICR
UUAUUAUGCG CGAGGCAUAU UG	HDI1R

- or a variant sequence which differs from any of the preceding sequences
  - either by addition to or removal from any of their respective extremities of one or several nucleotides,
  - or changing within any of said sequences of one or more nucleotides,
  - or both,

yet provided that in any of the above circumstances said probe still hybridizes with the same RNA or DNA target as the corresponding unmodified sequence.

A hybridization probe of the invention for detecting Bordetella pertussis strains contains :

- either a sequence belonging to a nucleic acid selected from the following group of nucleic acids and which includes itself of from 15 to the maximum number of nucleotides of the selected nucleic acid,

Group BPI1 :

CCACACCCAT CCTCTGGACA GGCTT	BPI1
AAGCCTGTCC AGAGGATGGG TGTGG	BPI1IC
AAGCCUGUCC AGAGGAUGGG UGUGG	BPI1ICR
CCACACCCAU CCUCUGGACA GGCUU	BPI1R

- or a variant sequence which differs from any of the preceding sequences
  - either by addition to or removal from any of their respective extremities of one or several nucleotides,
  - or changing within any of said sequences of one or more nucleotides,
  - or both,

yet provided that in any of the above circumstances said probe still hybridizes with the same RNA or DNA target as the corresponding unmodified sequence.

In the sequences given in groups NGI1, NMI1, NMI2, NMI3, NMI4, NMI5, BCI1, HDI1 and BPI1, the letters mean the following nucleotides :

A : Adenylic residue
C : Cytidylic residue
G : Guanidylic residue
T : Thymidylic residue
U : Uracylic residue

Under the expression "target" is meant a sequence complementary to any of the sequences of groups NGI1, NMI1, NMI2, NMI3, NMI4, BCI1, HDI1 and BPI1 as herein before defined.

This in case where the probe of the invention would comprise nucleic acid elongations on either side or both of said above defined sequences -- e.g. nucleic acid fragments of cloning vector or linker fragments resulting from the cleavage of said probe out of said cloning vector -- it is understood that such elongations should be selected such as to avoid the possibility that they could themselves hybridize with any other corresponding complementary nucleic acid sequence outside of the above target in a DNA of any micro-organism likely to be tested by the process of this invention as later defined. Such hybridization would be of a parasitical nature and reduce the specificity of the probe. Preferred probes consist of nucleic acid fragments formed of any of the sequences of the groups above defined, said fragments containing from 15 to the maximum number of nucleotides of the relevant nucleic acid sequence.

It is understood that in the above nucleotide sequences (and in the other ones referred to hereafter), the left end of the formulae always corresponds to a 5' extremity and the right end to a 3' extremity of the sequence concerned.

When reference is further made therein to a "probe of group 'X'" -- with 'X' from NGI1, NMI1, NMI2, NMI3, NMI4, NMI5, BCI1, HDI1 and BPI1 -- it should be understood that such probe has a sequence included in one of the nucleic acids belonging to that group as defined above or further defined hereafter.

It is also understood that the word "nucleotide" as used herein refers indistinctly to ribonucleotides and deoxyribonucleotides and modified nucleotides such as inosine unless otherwise specified. The expression "nucleotides" also encompasses those which further comprise modification groups, e.g. chemical modification groups which do not affect their hybridization capabilities fundamentally. Such modification groups aim, for instance, at facilitating their coupling, either directly or indirectly, with suitable markers or labels for the subsequent detection of the probes so marked or labeled particularly in their hybridization products with the relevant RNA or DNA strand, e.g. that or those initially contained in a biological sample together with other DNA(s) and/or RNA(s).

For instance, such modification groups are recognizable by antibodies which, in turn, can be recognized specifically by other antibodies, carrying a suitable enzymatic or fluorescent or chemiluminescent label. Possible labeling procedures will further be exemplified later herein.

The invention also relates to probes having any of the sequences defined above and in which some nucleotides are different, provided that the different nucleotide(s) do(es) not alter the specificity of the probes defined above. Some probes may consist of one of the nucleic acids belonging to any of the groups which are set forth above or of part thereof, said probes however including nucleotidic elongation on either sides thereof to the extent that such elongations do not alter the specificity of said probes with the genetic material of Neisseria gonorrhoeae, Neisseria meningitidis, Haemophilus ducreyi, Bordetella pertussis or Branhamella catarrhalis.

The invention thus provides for probes which are either replicas (those designated by numbers followed by "IC" or "ICR") in terms of nucleotide sequence of sequences contained in the RNAs or DNAs of most Neisseria gonorrhoeae, Neisseria meningitidis, Haemophilus ducreyi, Bordetella pertussis or Branhamella catarrhalis with occasionally a few insignificant differences in nucleotide sequences or formed of sequences, those designated by bare numbers or by numbers followed by "R", complementary to sequences included in the natural DNAs or RNAs of Neisseria gonorrhoeae, Neisseria meningitidis, Haemophilus ducreyi, Bordetella pertussis or Branhamella catarrhalis.

More particularly, it should be appreciated that the target sequences in the DNAs concerned consist in any of the following successive sequences present in most, if not all, Neisseria gonorrhoeae, Neisseria meningitidis, Haemophilus ducreyi, Bordetella pertussis or Branhamella catarrhalis strains, subject to possible insignificant natural differences from one strain to another, whereby such natural differences are not likely to affect the hybridization specificity of the probes of this invention with such targets :

In the case of Neisseria gonorrhoeae

GCGAAGTAGA ATAACGACGC ATCG

In the case of Neisseria meningitidis

CAGGGCGACG TCACACTTGA CC

GACGTCACAC TTGACCAAGA AC

GCACAGTAGA TAGCTATAAC GAACGC

TGCACAGTAG ATAGCAATAT CGAACGCA

AATGGAACAGAATCCATTCAGGGCGACGTCACACTTGACCAAGAACAAAA

In the case of Branhamella catarrhalis

CTTTGGTAAG ATGTTTAA

In the case of Haemophilus ducreyi

CAATATGCCT CGCGCATAAT AA

In the case of Bordetella pertussis

AAGCCTGTCC AGAGGATGGG TGTGG

The probes according to the invention can be formed by cloning of recombinant plasmids containing inserts including the corresponding nucleotide sequences, if need be cleaving the latter out from the cloned plasmids upon using the adequate nucleases and recovering them, e.g. by fractionation according to molecular weights. The probes according to the invention can also be synthesized chemically, for instance by the conventional phospho-triester method.

The invention also relates to a process for detecting Neisseria gonorrhoeae, Neisseria meningitidis, Haemophilus ducreyi, Bordetella pertussis or Branhamella catarrhalis strains in a biological sample, wherein said process comprises contacting said biological sample in which the nucleic acids (DNAs and RNAs) have been made accessible to hybridization, if need be under suitable denaturation conditions, with a probe of the invention under conditions enabling hybridization between the probe and complementary nucleic acids of the strains, which may be present in the sample, and detecting the hybrids possibly formed.

The process of the invention enables to discriminate Neisseria gonorrhoeae, Neisseria meningitidis, Haemophilus ducreyi, Bordetella pertussis or Branhamella catarrhalis from most other organism such as yeast, fungi, protozoa, other bacterial strains, human cells which are liable to the be present in the sample in which the organisms of interest are sought. The process relates to the detection of Neisseria gonorrhoeae, Neisseria meningitidis, Haemophilus ducreyi, Bordetella pertussis or Branhamella catarrhalis strains being directly in the sample of after the strain has been cultured.

The detection of a hybrid can be interpreted as meaning that an infection due to Neisseria gonorrhoeae, Neisseria meningitidis, Branhamella catarrhalis, Haemophilus ducreyi, or Bordetella pertussis was present in the biological sample, when any of the probes of groups NGI1, NMI1, NMI2, NMI3, NMI4, NMI5, BCI1, HDI1 and BPI1 is being used respectively.

According to an advantageous embodiment of the invention, in the process for detecting Neisseria gonorrhoeae, Neisseria meningitidis, Bordetella pertussis, Haemophilus ducreyi or Branhamella catarrhalis strains, the probes used are the ones hybridizing both with DNA globally and RNA of the Neisseria gonorrhoeae, Neisseria meningitidis, Bordetella pertussis, Haemophilus ducreyi or Branhamella catarrhalis strains, which may be present in the biological sample.

The hybridization conditions can be monitored relying upon several parameters, e.g. hybridization temperature, the nature and concentration of the components of the media, and the temperature under which the hybrids formed are washed.

The hybridization and wash temperature is limited in upper value, according to the probe (its nucleic acid composition, kind and length) and the maximum hybridization or wash temperature of the probes described herein is about 30° C to 55° C. At higher temperatures duplexing competes with the dissociation (or denaturation) of the hybrid formed between the probe and the target.

A preferred hybridization medium contains about 3 x SSC (1 x SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), about 25 mM of phosphate buffer pH 7.1, and 20% deionized formamide, 0.02% ficoll, 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone and about 0.1 mg/ml sheared denatured salmon

sperm DNA.

A preferred wash medium contains about 3 x SSC, 25 mM phosphate buffer pH 7.1 and 20% deionized formamide. Other hybridization or wash media can be used as well.

However, when modifications are introduced, be it either in the probes or in the media, the temperatures at which the probes can be used to obtain the required specificity, should be changed according to known relationships, such as those described in the following reference : B.D. HAMES and S.J. HIGGINS, (eds.). Nucleic acid hybridization. A practical approach, IRL Press, Oxford, U.K., 1985.

In this respect it should also be noted that, in general, DNA:DNA hybrids are less stable then RNA:DNA or RNA:RNA hybrids. Depending on the nature of the hybrid to be detected the hybridization conditions should be adapted accordingly to achieve specific detection.

The process for detecting Neisseria gonorrhoeae, Neisseria meningitidis, Bordetella pertussis, Haemophilus ducreyi or Branhamella catarrhalis strains generally, according to the invention can be carried out by suitably adjusting the hybridization temperature to a value at which hybridization is specific, and in such a case washing under more stringent conditions is not necessary.

According to another embodiment of the process of the invention, the hybridization temperature needs not necessarily be adjusted to the value at which hybridization is specific and in particular can be lower than the temperature at which hybridization is specific, provided washing is carried out at a temperature corresponding to the value at which hybridization is specific.

In a process embodiment for detecting Neisseria gonorrhoeae strains (and for distinguishing them from other bacterial taxa) with a probe of group NGI1, the hybridization temperature and/or the wash temperature is suitably adjusted to about 50° C, the media being those above defined.

In a process embodiment for detecting Neisseria meningitidis strains (and for distinguishing them from other bacterial taxa) with a probe of group NMI1, the hybridization temperature and/or the wash temperature is suitably adjusted to about 45° C, the media being those above defined.

In a process embodiment for detecting Neisseria meningitidis strains (and for distinguishing them from other bacterial taxa) with a probe of group NMI2, the hybridization temperature and/or the wash temperature is suitably adjusted to about 45° C, the media being those above defined.

In a process embodiment for detecting Neisseria meningitidis strains (and for distinguishing them from other bacterial taxa) with a probe of group NMI3, the hybridization temperature and/or the wash temperature is suitably adjusted to about 40° C, the media being those above defined.

In a process embodiment for detecting Neisseria meningitidis strains (and for distinguishing them from other bacterial taxa) with a probe of group NMI4, the hybridization temperature and/or the wash temperature is suitably adjusted to about 48° C, the media being those above defined.

In a process embodiment for detecting Neisseria meningitidis strains (and for distinguishing them from other bacterial taxa) with a probe of group NMI5, the hybridization temperature and/or the wash temperature is suitably adjusted to about 58° C, the media being those above defined.

In a process embodiment for detecting Branhamella catarrhalis strains (and for distinguishing them from other bacterial taxa) with a probe of group BCI1, the hybridization temperature and/or the wash temperature is suitably adjusted to about 30° C, the media being those above defined.

In a process embodiment for detecting Bordetella pertussis strains (and for distinguishing them from other bacterial taxa) with a probe of group BPI1, the hybridization temperature and/or the wash temperature is suitably adjusted to about 55° C, the media being those above defined.

In a process embodiment for detecting Haemophilus ducreyi strains (and for distinguishing them from other bacterial taxa) with a probe of group HDI1, the hybridization temperature and/or the wash temperature is suitably adjusted to about 40° C, the media being those above defined.

The invention further relates to a kit for detecting specifically Neisseria meningitidis strains containing :

- a probe specific for Neisseria meningitidis i.e. a probe of group NMI1, NMI2, NMI3, NMI4 or NMI5;
- the buffer or components necessary for producing the buffer enabling an hybridization reaction between these probes and only the DNAs and/or RNAs of a strain of Neisseria meningitidis to be carried out,
- when appropriate means for detecting the hybrids resulting from the proceeding hybridization.

The invention further relates to a kit for detecting specifically Neisseria gonorrhoeae strains containing :

- a probe specific for Neisseria gonorrhoeae i.e. a probe of group NGI1;
- the buffer or components necessary for producing the buffer enabling an hybridization reaction between these probes and only the DNAs and/or RNAs of a strain of Neisseria gonorrhoeae to be carried out,
- when appropriate means for detecting the hybrids resulting from the proceeding hybridization.

The invention further relates to a kit for detecting specifically Branhamella catarrhalis strains containing :

- at least one probe selected among any of those that are specific for Branhamella catarrhalis as above defined, i.e. a probe of group BCI1;
- the buffer or components necessary for producing the buffer enabling an hybridization reaction between these probes and only the DNAs and/or RNAs of a strain of Branhamella catarrhalis to be carried out,
- when appropriate means for detecting the hybrids resulting from the proceeding hybridization.

The invention further relates to a kit for detecting specifically Haemophilus ducreyi strains containing :

- at least one probe selected among any of those that are specific for Haemophilus ducreyi as above defined, i.e. a probe of group HDI1;
- the buffer or components necessary for producing the buffer enabling an hybridization reaction between these probes and only the DNAs and/or RNAs of a strain of Haemophilus ducreyi to be carried out,
- when appropriate means for detecting the hybrids resulting from the proceeding hybridization.

The invention further relates to a kit for detecting specifically Bordetella pertussis strains containing :

- at least one probe selected among any of those that are specific for Bordetella pertussis as above defined, i.e. a probe of group BPI1;
- the buffer or components necessary for producing the buffer enabling an hybridization reaction between these probes and only the DNAs and/or RNAs of a strain of Bordetella pertussis to be carried out,
- when appropriate means for detecting the hybrids resulting from the proceeding hybridization.

The probes of the invention can be used in a sandwich hybridization system which enhances the specificity of a nucleic acid probe based assay. The principle and the use of sandwich hybridizations in a nucleic acid probe based assay have been already described (e.g.: DUNN and HASSEL, Cell 12 : 23-36; 1977; RANKI et al., Gene, 21: 77-85; 1983). Although direct hybridization assays have favorable kinetics, sandwich hybridizations are advantageous with respect to a higher signal to noise ratio. Moreover sandwich hybridizations can enhance the specificity of a nucleic acid probe based assay.

If properly designed, a sandwich hybridization assay indeed maximizes the specificity of a nucleic acid probe based test when using two probes recognizing two different nucleic acid stretches of one and the same organism. The only demands which must be met are that both probes (i) hybridize to the same nucleic acid molecule of the target organism and (ii) do not hybridize to the same non-target organisms.

For two given probes I and II, the sandwich hybridization system can be described as follows :
Probe n° I hybridizes to nucleic acid from organisms A and B (not with C);
Probe n° II hybridizes to nucleic acid from organisms A and C (not with B).

Since it is absolutely required that both probes hybridize to the target nucleic acid, a detectable signal will be generated only if nucleic acid from organism A is present in the sample. It is obvious that if one of the probes is specific for the organism to be detected, the other probe can be composed of any specific or non-specific sequence provided that it hybridizes to the same target molecule than the first probe.

The probes of the invention -- groups NGI1, NMI1, NMI2, NMI3, NMI4, NMI5, BCI1, HDI1, and BPI1 -- can be used in a sandwich hybridization assay which is specific for Haemophilus ducreyi, Branhamella catarrhalis, Neisseria gonorrhoeae, Neisseria meningitidis or Bordetella pertussis respectively in combination with another, non-specific or specific, probe hybridizing to the same target molecule.

In the sandwich hybridization process the probes can be added simultaneously or not, to the biological sample in which the target DNA or RNA is sought.

The invention also relates to a kit for sandwich hybridization assay, for the detection in vitro of Neisseria gonorrhoeae, Neisseria meningitidis, Bordetella pertussis , Haemophilus ducreyi or Branhamella catarrhalis strains in a biological sample, said kit containing :

- at least one of the probes or one of the combinations of probes specific for the organisms of interest as above defined,
- the buffer or components necessary for producing the buffer enabling hybridization reaction between these probes and the DNAs and/or RNAs of a strain of Neisseria gonorrhoeae, Neisseria meningitidis, Bordetella pertussis , Haemophilus ducreyi or Branhamella catarrhalis to be carried out,
- when appropriate means for detecting the hybrids resulting from the preceding hybridization.

The probes of the invention can be used also in a competition hybridization protocol.

In a competition hybridization the target molecule competes with the hybrid-formation between a specific probe and its complement. The more target is present, the lower the amount of hybrid formed between the probe and its complement. A positive signal, which indicates that the specific target was present, is seen by a decrease in hybridization reaction as compared to a system to which no target was added. In a particular embodiment the specific oligonucleotide probe, conveniently labeled, is hybridized

with the target molecule. Next, the mixture is transferred to a recipient (e.g. a microtiter dish well) in which a oligonucleotide complementary to the specific probe is fixed and the hybridization is continued. After washing the hybrids between the complementary oligonucleotide and the probe are measured, preferably quantitatively, according to the label used.

The oligonucleotides of the invention can be used either as amplification primers in the polymerase chain reaction technique (PCR; Mullis and Faloona, Methods in Enzymology 155 :335- , 1987.) to generate specific enzymatically amplified fragments and/or as probes to detect fragments amplified between bracketing oligonucleotide primers.

The specificity of a PCR-assisted hybridization assay can be controlled at different levels.

The amplification process or the detection process or both can be specific. The latter case, giving the highest specificity, is preferred. Such a highly specific PCR-assisted test can be developed using the probes of the invention.

However, in some occurrences, a non-specific amplification process, using conserved primers bracketing the detection-probes of the invention, coupled to a specific detection, might be advantageous in order to standardize the amplification process in such a way that it can be used for a great variety of organisms. Amplification primers to be used in a standardized amplification process can be found in the conserved region of the 16S and 23S rRNA gene flanking the spacer region (see example 1).

In most of the cases the number of specific probes for the organisms of interest which can be derived from the spacer regions is not limited to the probes described in this text.

For most organisms only one probe is described to demonstrate the feasibility of spacer regions for the development of highly specific and sensitive probes for a variety of bacteria. The only exception is Bordetella pertussis for which only one particular region (from nucleotide 271 to 299 in the Bordetella pertussis sequence; Fig. 2, top line) of the spacer region has a specific sequence. However, from the spacer region sequence of Bordetella pertussis probes may be devised which can be valuable in the simultaneous detection of highly related Bordetella species. Probes which detect Bordetella species other than Bordetella pertussis, may also be deduced from the sequences disclosed in Fig. 2. Likewise, potentially specific probes for Moraxella nonliquefaciens may be inferred from the spacer-region sequence shown in Fig. 7.

CONDITIONS OF THE USE OF PROBES :

The probes of the invention are advantageously labeled. Any conventional label can be used. The probes can be labeled by means of radioactive tracers such as ^{32}P, ^{35}S, ^{125}I, ^{3}H and ^{14}C.

The radioactive labeling can be carried out according to any conventional method such as terminal labeling at the 3' or 5' position with the use of a radio-labeled nucleotide, a polynucleotide kinase (with or without dephosphorylation by a phosphatase), a terminal transferase, or a ligase (according to the extremity to be labeled). One of the probes of the invention can be the matrix for the synthesis of a chain consisting of several radioactive nucleotides or of several radioactive and non radioactive nucleotides.

The probes of the invention can also be prepared by a chemical synthesis using one or several radioactive nucleotides. Another method for radioactive labeling is a chemical iodination of the probes of the invention which leads to the binding of several ^{125}I atoms on the probes.

If one of the probes of the invention is made radioactive to be used for hybridization with a non radioactive RNA or DNA, the method of detecting hybridization will depend on the radioactive tracer used. Generally, autoradiography, liquid scintillation, gamma counting or any other conventional method enabling one to detect an ionizing ray issued by the radioactive tracer can be used.

Non radioactive labeling can also be used by associating the probes of the invention with residues having : immunological properties (e.g. antigen or hapten) a specific affinity for some reagents (e.g. ligand) properties providing a detectable enzymatic reaction (e.g. enzyme, co-enzyme, enzyme substrate or substrate taking part in an enzymatic reaction) or physical properties such as fluorescence or emission or absorption of light at any wave length. Antibodies which specifically detect the hybrids formed by the probe and the target can also be used.

A non-radioactive label can be provided when chemically synthesizing a probe of the invention, the adenosine, guanosine, cytidine, thymidine and uracyl residues, thereof being liable to be coupled to other chemical residues enabling the detection of the probe or the hybrids formed between the probe and a complementary DNA or RNA fragment.

However, the nucleotidic sequence of the probe when modified by coupling one or more nucleotides to other chemical residues, would be the same as the nucleotide sequence of one of the probes of the invention.

The invention also relates to processes for detecting by hybridization RNA and/or DNA with the probes of the invention, which have been labeled and can be detected as described above. In this regard, conventional methods of hybridization can be used.

For detecting cells coming from or being themselves living organisms, the RNA and/or DNA of these cells if need be, is made accessible by partial or total lysis of the cells, using chemical or physical processes, and contacted with one or several probes of the invention which can be detected. This contact can be carried out on an appropriate support such as a nitrocellulose, cellulose, or nylon filter in a liquid medium or in solution. This contact can take place under sub-optimal, optimal conditions or under restrictive conditions (i.e. conditions enabling hybrid formation only if the sequences are perfectly homologous on a length of molecule). Such conditions include temperature, concentration of reactants, the presence of substances lowering the optimal temperature of pairing of nucleic acids (e.g. formamide, dimethylsulfoxide and urea) and the presence of substances apparently lowering the reaction volume and/or accelerating hybrid formation (e.g. dextran sulfate, polyethyleneglycol or phenol).

The elimination of probe of the invention which has not hybridized can be carried out by washing with a buffer solution of appropriate ionic strength and at an appropriate temperature, with or without treatment with S1 nuclease or any other enzyme digesting single strand DNA or RNA but not digesting DNA-RNA hybrids or double strand DNA.

In a liquid medium, the hybrids of the probe of the invention paired to the cellular DNA or RNA fragments can be separated from the rest of the liquid medium in different ways, e.g. by chromatography over hydroxyapatite.

Then the hybridized probes are detected by means of the label on the probe.

In order to target the chromosomal DNA fragments, after treating RNA by one or several enzymes and denaturation of DNA fragments (i.e. separation of both chains), one of the probes of the invention is contacted with the DNA fragments under the conditions enabling hybridization and after the time necessary to get to the end of the hybridization, the non-hybridized fragments are separated from the hybridized fragments and the label is detected as it has been described above for the detection of the cells.

Generally speaking, the different probes of the invention can also be contained in recombinant DNA enabling their cloning, if the presence of a heterologous DNA is not a nuisance for the specificity of the probes in the encompassed uses.

In Fig.1 to 7 alignments of spacer regions (completely or partially sequenced) found in various microorganisms are shown. Matches and gaps are indicated by ":" and "-" respectively. For all sequences the noncoding strand is shown in its 5'-3' orientation.

The 5'-end is proximal to the 16S rRNA gene, the 3'-end proximal to the 23S rRNA gene.

In Fig.1 the nucleic acid sequence alignment of the 16S rRNA proximal end of the spacer region between the 16S and 23S rRNA gene of Neisseria gonorrhoeae strains NCTC 8375 (top line) and ITM 4367 (bottom line) is shown.

In Fig.2 the nucleic acid sequence alignment of the spacer region between the 16S and 23S rRNA of Bordetella pertussis ATCC 10380 (top line) and Bordetella bronchiseptica NCTC 452 (bottom line) is shown.

In Fig.3 the nucleic acid sequence alignment of the spacer region between the 16S and 23S rRNA of Neisseria meningitidis NCTC 10025 (top line) and Neisseria gonorrhoeae NCTC 8375 (bottom line) is shown.

In Fig.4 the nucleic acid sequence alignment of the spacer region between the 16S and 23S rRNA of Neisseria gonorrhoeae NCTC 8375 (top line) and Bordetella pertussis ATCC 10380 (bottom line) is shown.

In Fig.5 the nucleic acid sequence alignment of the spacer region between the 16S and 23S rRNA of Branhamella catarrhalis ITM 4197 (top line) and Neisseria gonorrhoeae NCTC 8375 (bottom line) is shown.

In Fig.6 the nucleic acid sequence alignment of the spacer region between the 16S and 23S rRNA of Haemophilus ducreyi CIP 542 (top line) and Escherichia coli (bottom line) is shown.

In Fig. 7 the nucleic acid sequence alignment of the spacer region between the 16S and 23S rRNA of Branhamella catarrhalis ITM 4197 (top line) and Moraxella nonliquefaciens ATCC 19975 (bottom line) is shown.

It is to be pointed out that each nucleic acid sequence of the spacer region between the 16S and 23S rRNA genes of respectively each organism referred to in figures 1 to 7 (except the one of E. coli) is new.

The strains used can be obtained at the respective culture collections :

ATCC : American Type Culture Collection, Rockville, Md., USA.
CIP : Collection de l'Institut Pasteur, Paris, France.
ITM : Institute of Tropical Medicine, Antwerp, Belgium.
NCTC : National Collection of Type Cultures, Central Public Health Laboratory, London, United Kingdom.

The examples hereafter relate to the preparation of the probes of the invention and the experimental results with respect to the specificity and sensitivity of the probes. The following organisms of clinical relevance were studied : Neisseria gonorrhoeae, Neisseria meningitidis, Bordetella pertussis, Haemophilus ducreyi and Branhamella catarrhalis.

The examples illustrate that species specific and highly sensitive probes could readily be found in the spacer region of all organisms studied. Moreover it is shown that probes could be constructed from this region for organisms for which no species specific and highly sensitive probe could be found in the 16S and/or 23S rRNA molecule.

The methods used are essentially the same as described by ROSSAU et al., J. Gen. Microbiol.; 135 : 1735-1745, 1989; or in the European patent application n° 8940/045.3 unless otherwise stated. All methods used, except the enzymatical amplification of rRNA gene fragments, are currently known to people skilled in the art. The enzymatical amplification of rRNA gene fragments spanning the 16S-23S rRNA spacer region were obtained by the polymerase chain reaction technique (PCR) performed according to the recommendations given in the "Gene Amp" kit of Perkin Elmer Cetus. As PCR primers oligonucleotides corresponding to conserved or semi-conserved regions in the rRNA molecules were used.

EXAMPLE 1

Neisseria meningitidis and Neisseria gonorrhoeae both are important human pathogens, responsible for meningitis and gonorrhea respectively. These organisms are extremely highly related and their differentiations from each other and other Neisseria species is error-prone. DNA probes specific for Neisseria meningitidis and Neisseria gonorrhoeae may aid in the correct differentiation between both Neisseria species and may be used for direct detection of these species in clinical samples.

A number of DNA probes have been described for the detection of Neisseria gonorrhoeae (European Patent Application nr 0272 009 and 0337 896; URDEA et al., Clin. Chem. 35 : 1571-1575, 1989; TOTTEN et al., J. Infect. Dis. 148 : 462-471, 1989; DONEGAN et al., Mol. Cell. Probes 3 : 13-26, 1989; KOLBERG et al., Mol. Cell. Probes 3 : 59-72, 1989.). However, some of these probes were found to cross-react with non-Neisseria gonorrhoeae strains or were not highly sensitive. None of these probes were derived from the 16S-23S rRNA spacer region.

A DNA probe which detects Neisseria meningitidis strains has also been described (KOLBERG et al., Mol. Cell. Probes 3 : 59-72, 1989). This probe, devised from the pilin gene of Neisseria gonorrhoeae, was neither highly specific nor highly sensitive for Neisseria meningitidis.

The sequence of the spacer region between the 16S and 23S rRNA gene of the type strains of Neisseria gonorrhoeae and Neisseria meningitidis was determined using cloned material originating from a PCR-fragment spanning the spacer region. The alignment of both sequences, shown in Fig. 3, revealed several potential probe sequences.

An unexpected inserted sequence of about 60 basepairs was detected in the spacer region of the Neisseria meningitidis strain. Oligonucleotides with the following sequences were derived from this inserted sequence :

GGTCAAGTGT	GACGTCGCCC	TG	NMI1
GTTCTTGGTC	AAGTGTGACG	TC	NMI2

Also in another area of the spacer region (from basepairs 365 to 386 in the Neisseria meningitidis sequence in Fig. 3) a substantial degree of mismatch was revealed between Neisseria meningitidis and Neisseria gonorrhoeae. From this area two oligonucleotide probes (NMI3 and NGI1 for the detection of Neisseria meningitidis and Neisseria gonorrhoeae respectively) were chemically synthesized:

GCGTTCGTTA	TAGCTATCTA	CTGTGC	NMI3
CGATGCGTCG	TTATTCTACT	TCGC	NGI1

These oligonucleotides were ^{32}P-labeled at their 5'ends using polynucleotide kinase or tailed at their 3'ends with digoxigeninated UTP using terminal transferase and used as hybridization probes. As target dot-spotted denatured genomical DNA of a great number of Neisseria meningitidis and Neisseria gonorrhoeae

strains from different locations and several strains of other bacterial taxa was used.

The hybridization-mixture was either 3 x SSC, 25 mM potassium phosphate buffer, pH 7, deionized formamide (20%, v/v), Ficoll (0.02%, w/v), bovine serum albumin (0.02%, w/v), polyvinylpyrrolidone (0.02%, w/v) and sheared, denatured salmon sperm DNA (0.1 mg ml-1) or the solution given in the protocol sheet of the DNA labeling and detection kit nonradioactive (Boehringer Mannheim) except that 3 x SSC (1 x SSC is : 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) in stead of 5 x SSC was used and formamide was added up to 20% (v/v). The wash-solution contained 3 x SSC, 20% formamide and 25 mM phosphate buffer pH 7.1.

The hybridization results are summarized in the table below. The hybridization- and wash-temperature for each probe is indicated between parenthesis. All probes tested proved to be highly specific and highly sensitive for Neisseria gonorrhoeae (probe NGI1) or Neisseria meningitidis (probes NMI1, NMI2 and NMI3).

TAXON	Nr of strains positive/Nr of strains tested			
	NMI1 (45°C)	NMI2 (45°C)	NMI3 (40°C)	NGI1 (50°C)
Neisseria meningitidis	52/53	10/11	56/56	0/11
Neisseria sp ATCC 43831	1/1	1/1	1/1	0/1
Neisseria gonorrhoeae	0/16	0/9	0/10	10/10
Neisseria polysaccharea	0/3	-	0/3	0/3
Neisseria lactamica	0/10	-	0/10	0/10
Neisseria cinerea	0/4	-	0/4	2/4
Neisseria mucosa	0/3	-	0/3	0/3
Neisseria macacae	0/1	-	0/1	0/1
Neisseria flavescens	0/1	-	0/1	0/1
Neisseria subflava	0/2	-	0/2	0/2
Neisseria sicca	0/1	-	0/1	0/1
Neisseria elongata	0/2	-	0/2	0/2
Neisseria canis	0/1	-	0/1	0/1
Neisseria animalis	0/1	-	0/1	0/1
Neisseria denitrificans	0/1	-	0/1	0/1
Neisseria sp	0/5	-	0/4	0/3
CDC group M-5	0/1	-	0/1	0/1
CDC group EF-4a	0/1	-	0/1	0/1
Kingella denitrificans	0/2	-	0/1	0/1
Kingella kingae	0/1	-	0/1	0/1
Simonsiella muelleri	0/1	-	0/1	0/1
Simonsiella crassa	0/1	-	0/1	0/1
Simonsiella steedae	0/1	-	0/1	0/1
Simonsiella sp	0/1	-	0/1	0/1
Alysiella filiformis	0/1	-	0/1	0/1
Eikenella corrodens	0/2	-	0/2	0/2
Chromobacterium violaceum	0/1	-	0/1	0/1
Iodobacter fluviatile	0/1	-	0/1	0/1
Aquaspirilum dispar	0/1	-	0/1	0/1
Comamonas testosteroni	0/1	-	0/1	0/1
Haemophilus influenzae	0/1	-	-	-
Haemophilus ducreyi	0/1	-	0/1	0/1
Kingella indologenes	0/1	-	0/1	0/1
Moraxella lacunata	0/1	-	-	-
Moraxella nonliquefaciens	0/1	-	-	-
Moraxella catarrhalis	0/3	-	0/2	0/2
Moraxella cuniculi	0/1	-	-	-
Moraxella caviae	0/1	-	-	-
Moraxella ovis	0/1	-	-	-
Moraxella osloensis	0/1	-	-	-
Escherichia coli	0/1	0/1	0/1	0/1

The specificity of the detection with probes NMI3 and NGI3 was also checked after enzymatical amplification of the spacer regions with the following amplification primers :

TGGGTGAAGTCGTAACAAGGTA AP16

CAC GTC CTTCGTCGCCT AP23

located at the 3' end of the 16S rRNA gene and the 5' end of the 23S rRNA gene respectively. Hundred nanogram of genomical DNA of a strain of Neisseria gonorrhoeae, Neisseria meningitidis, Haemophilus ducreyi, Bordetella pertussis and Branhamella catarrhalis was used in the PCR reaction. After amplification, 1/10 of the yield was loaded on an agarose gel, electrophoresed and blotted on a nylon membrane.

The membrane was consecutively hybridized with the probes NGI1 and NMI3.

Significant hybridization signals could only be detected in lanes where Neisseria gonorrhoeae or Neisseria meningitidis material was present when NGI1 or NMI3 were used as probes respectively.

EXAMPLE 2 :

Bordetella pertussis is the causative agent of whooping cough. Due to vaccination-programs the disease has become a minor problem in the industrialized countries.

However, in third-world countries pertussis is a leading cause of childhood mortality.

Strains of three Bordetella species (Bordetella pertussis, Bordetella parapertussis and Bordetella bronchiseptica) are extremely highly related (KLOOS et al., Int.J.Syst. Bacteriol. 31 : 173-176, 1981; DE LEY et al., Int. J. Syst. Bacteriol. 36 : 405-414, 1986) and should be considered as belonging to one genospecies. This genotypical relationship is also reflected in many other characteristics of these bacteria making their phenotypical differentiation tedious.

Clinical signs of pertussis often are atypical and laboratory diagnosis is needed. As yet, no sensitive, specific and rapid test exists. Culture still is the method of choice, but recovery rates are low and the results usually are available only 3 to 7 days after inoculation (FRIEDMAN, Clin. Microbiol. Rev. 4 : 365-376, 1988; HALPERIN et al., J. Clin. Microbiol. 27 : 752-757, 1989). A DNA-probe based assay may greatly improve the diagnosis of Bordetella pertussis infections.

Probes for the detection of Bordetella pertussis are described in the literature (PARK et al., FEMS Microbiol. Lett. 52 : 19-24, 1988; McPHEAT and McNALLY, J. Gen. Microbiol. 133 : 323-330, 1987 and FEMS Microbiol. Lett. 41 : 357-360, 1987; McLAFFERTY et al., Abstracts of the Annual Meeting of the American Society for Microbiology C-168, 1986, and C-322, 1987.). The probe described by McLAFFERTY et al. (1986 and 1987) is not highly specific. For the other probes described the data presented are to scanty to infer the degree of specificity and sensitivity.

Part of the ribosomal RNA gene of the following strains were enzymatically amplified and cloned in a plasmid vector : Bordetella pertussis ATCC 10380, Bordetella parapertussis NCTC 5952 (type strain), and Bordetella bronchiseptica NCTC 452 (type strain). The cloned fragments of the different species were partially sequenced using the dideoxy chain termination method and their sequences were compared. The sequences information of the 16S rRNA gene which came available, indicated that no species specific probes could be devised (ROSSAU et al., unpublished). However, as shown in the alignment in Fig. 2, a non-homologous area (from basepairs 271 to about 300) was found in the spacer region between the 16S and 23S rRNA genes of the Bordetella pertussis and the Bordetella bronchiseptica strain.

The sequence of the spacer region of the Bordetella parapertussis strain was virtually identical to the Bordetella bronchiseptica sequence (ROSSAU et al., unpublished).

From the area between nucleotide 271 and 295 in the spacer region of Bordetella pertussis a oligonucleotide probe with the following sequence was derived :

CCACACCCAT CCTCTGGACA GGCTT BPI1

The oligonucleotide probe was chemically synthesized and labeled with digoxigenine - UTP using terminal transferase. The results obtained with dot-spotted denatured genomical DNA as target are summarized in the table below.

TAXON	Hybridization with BPI1 at 55°C Nr of strainspositive/Nr of strains tested
Bordetella pertussis	4/4
Bordetella parapertussis	0/3
Bordetella bronchiseptica	0/3
Alcaligenes denitrificans	0/1
Alcaligenes paradoxus	0/1
Oligella ureolytica	0/1
Oligella urethralis	0/1
Taylorella equigenitalis	0/1
Pseudomonas cepacia	0/1
Pseudomonas solanacearum	0/1
Comamonas testosteroni	0/1
Neisseria meningitidis	0/1
Branhamella catarrhalis	0/1
Haemophilus influenzae	0/1

Under the conditions used the probe BPI1 proved to be 100% specific and 100% sensitive for Bordetella pertussis.

The hybridization-mixture was as described in the protocol sheet of the DNA labeling and detection kit nonradioactive (Boehringer Mannheim) except that 3 x SSC (1 x SSC is : 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) instead of 5 x SSC was used and formamide was added up to 20% (v/v). The wash-solution contained 3 x SSC, 20% formamide and 25 mM phosphate buffer pH 7.1. The hybridization and wash temperature was 55° C.

EXAMPLE 3 :

Branhamella catarrhalis, also known as Moraxella catarrhalis or Neisseria catarrhalis, is a fastidious, biochemically rather inert bacterium. Recently its important pathogenic potential was recognized. Branhamella catarrhalis seems to be frequently involved in serious infections of the respiratory tract (HAGER et al., Rev. Infect. Dis. 9 : 1140-1149, 1987). The diagnosis of Branhamella catarrhalis requires culture of the organism, which may be hampered by overgrowth by less fastidious micro-organisms, and a battery of phenotypical tests to distinguish this organisms from commensals, such as Neisseria species, present in the oral cavity.

In some occurrences the phenotypical test are inconclusive about the identity of the presumptive Branhamella catarrhalis isolate, since there only are a limited number of tests which differentiate Branhamella catarrhalis from phenotypical similar bacteria (RIOU and GUIBOURDENCHE, Drugs 31 [suppl.3] : 1-6, 1986). The use of a DNA probe based assay may considerably simplify the laboratory diagnosis of Branhamella catarrhalis. A DNA probe for Branhamella catarrhalis derived from an unspecified DNA fragment and which cross-hybridized with DNA from Neisseria caviae was described by BEAULIEU and ROY (Abstracts of the Annual Meeting of the American Society for Microbiology, Abstract nr D-249, 1989).

Part of the rRNA gene of Branhamella catarrhalis ITG 4197 was enzymatically amplified by the

polymerase chain reaction technique and cloned in a plasmid vector. The fragment spanning the 16S-23S rRNA spacer region was subsequently sequenced by the dideoxy chain termination technique. The sequence is shown in Fig. 7 (top line). From the sequence data the following oligonucleotide was selected and chemically synthesized :

TATCAGAAGC AAGCTTCCTA ACTTCGTT BCI1

The oligonucleotide was ^{32}P-labeled at its 5' end with polynucleotide kinase and used as a hybridization probe. As target dot-spotted denatured genomical DNA of 31 Branhamella catarrhalis strains from different locations and 19 strains of other bacterial taxa was used.

The hybridization-mixture was either 3 x SSC (1 x SSC is : 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), 25 mM potassium phosphate buffer, pH 7, deionized formamide (20%, v/v), Ficoll (0.02%, w/v), bovine serum albumin (0.02%, w/v), polyvinylpyrrolidone (0.02%, w/v) and sheared, denatured salmon sperm DNA (0.1 mg ml-1). The wash-solution contained 3 x SSC, 20% formamide and 25 mM phosphate buffer pH 7.1. The hybridization and wash temperature was 30 ° C.

Under the conditions used probe BCI1 hybridized to all Branhamella catarrhalis strains.

None of the strains tested belonging to other bacterial species gave a significant hybridization signal with the probe.

The non-Branhamella catarrhalis strains tested are :

Moraxella lacunata	ATCC 17967
Moraxella lacunata	ATCC 17952
Moraxella bovis	ITM 1601
Moraxella nonliquefaciens	ATCC 19975
Neisseria cuniculi	ITM 3388
Neisseria ovis	NCTC 11227
Neisseria caviae	ATCC 14659
Alysiella sp.	ATCC 29468
Moraxella osloensis	LMG 1043
Moraxella osloensis	ATCC 17974
"Moraxella paraphenylpyruvica"	LMG 5125
"Moraxella camembertii"	LMG 7022
Psychrobacter immobilis	LMG 6784
Acinetobacter calcoaceticus	ATCC 23055

Escherichia coli	B
Haemophilus influenzae	NCTC 8143
Eikenella corrodens	NCTC 10596
Xanthomonas maltophilia	LMG 958
Xanthomonas campestris	LMG 568

EXAMPLE 4 :

Haemophilus ducreyi, the causative agent of chancroid, is a fastidious Gram-negative bacterium. The

culture of this organism is both difficult and insensitive; yet it still is the method of choice for the diagnosis of Haemophilus ducreyi infections. The use of highly specific probes may obviate the culture and increase the sensitivity of the diagnosis. Cloned DNA-probes for Haemophilus ducreyi, showing weak cross-reactivity with other Haemophilus and Pasteurella species, and targeting genes coding for proteins were described by PARSONS et al. (J. Clin. Microbiol. 27 : 1441-1445, 1989).

Part of the rRNA gene of the type strain of Haemophilus ducreyi CIP 542 was enzymatically amplified by the polymerase chain reaction and cloned in a plasmid vector.

The sequence of the spacer region between the 16S and 23S rRNA gene was obtained by the dideoxy chain termination technique. From the nucleic acid sequence the following oligonucleotide was selected and chemically synthesized :

CACCCTTTAA TCCGAAGATA TTACG HDI1

The oligonucleotide was ^{32}P-labeled at its 5' ends or tailed at its 3' ends with digoxigeninated UTP using terminal transferase and used as a hybridization probe.

As target dot-spotted denatured genomical DNA of 41 Haemophilus ducreyi strains from different locations and several strains of other bacterial taxa was used. The oligonucleotide probe hybridized exclusively to all Haemophilus ducreyi strains tested.

The hybridization-mixture was either 3 x SSC, 25 mM potassium phosphate buffer, pH 7, deionized formamide (20%, v/v), Ficoll (0.02%, w/v), bovine serum albumin (0.02%, w/v), polyvinylpyrrolidone (0.02%, w/v) and sheared, denatured salmon sperm DNA (0.1 mg ml-1) or the solution given in the protocol sheet of the DNA labeling and detection kit nonradioactive (Boehringer Mannheim) except that 3 x SSC (1 x SSC is : 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) in stead of 5 x SSC was used and formamide was added up to 20% (v/v). The wash-solution contained 3 x SSC, 20% formamide and 25 mM phosphate buffer pH 7.1. The hybridization and wash temperature was 40 °C.

The non-Haemophilus ducreyi strains tested were :
Escherichia coli MC 1061
Escherichia coli B
Actinobacillus actinomycetemcomitans NCTC 9710
Actinobacillus lignieresii NCTC 4189
Haemophilus aphrophilus NCTC 5906
Haemophilus influenzae NCTC 8143
Histophilus ovis HIM 896-7
Pasteurella multocida NCTC 10322
Branhamella catarrhalis ITM 4197
Comamonas testosteroni ATCC 17407
Oligella urethralis LMG 6227
Neisseria gonorrhoeae ITM 4437
Campylobacter jejuni CCUG 11284
Acinetobacter calcoaceticus ATCC 23055
Unidentified strain ITM 3565

Claims

1. Probe consisting of at least about 15 oligonucleotides of the spacer region between rRNA genes of a non viral organism, particularly prokaryotic organism and more particularly bacteria, and preferably from about 15 oligonucleotides to about the maximum number of oligonucleotides of the spacer region and more preferably from about 15 to about 100 oligonucleotides.

2. Probe according to claim 1, for use in a hybridization assay, liable to be obtained in the process which comprises constructing an oligonucleotide that is sufficiently complementary to hybridize to a sequence of the spacer region between rRNA genes, particularly the spacer region between the 16S rRNA gene and the 23S rRNA gene, selected to be unique to non-viral organisms, particularly prokaryotic organisms, more particularly bacteria, sought to be detected, said sequence of the spacer region between rRNA genes being selected
   - either by
     * comparing the nucleotide sequence of the spacer region between the rRNA genes of the

sought organism with the nucleotide sequence of the spacer region between the rRNA genes of the closest neighbours,

* selecting a sequence of at least 15 oligonucleotides, and preferably from about 15 to about the maximum number of oligonucleotides of the spacer region, and more preferably from about 15 to about 100 oligonucleotides of the spacer region between rRNA genes of the sought organism which presents at least one mismatch with the spacer region between the rRNA genes of at least one of the closest neighbours,

- or by
  * deleting, in the spacer region between the rRNA genes of the organism to be sought, the tRNA genes and possibly the signal sequences, to obtain a shortened spacer region and
  * determining by trial and error a specific nucleotide sequence of at least about 15 oligonucleotides, and preferably from about 15 to about the maximum number of oligonucleotides of the spacer region, and more preferably from about 15 to about 100 oligonucleotides, from the shortened spacer region, said sequence being able to hybridize specifically with the nucleic acids (DNA and/or RNAs) of the sought organism.

3. Probe according to anyone of claims 1 or 2, containing
   - either a sequence belonging to a nucleic acid selected from the following group of nucleic acids and which includes itself of from 15 to the maximum number of nucleotides of the selected nucleic acid :

Group NGI1 :			
CGATGCGTCG	TTATTCTACT	TCGC	NGI1
GCGAAGTAGA	ATAACGACGC	ATCG	NGI1IC
GCGAAGUAGA	AUAACGACGC	AUCG	NGI1ICR
CGAUGCGUCG	UUAUUCUACU	UCGC	NGI1R
Group NMI1 :			
GGTCAAGTGT	GACGTCGCCC	TG	NMI1
CAGGGCGACG	TCACACTTGA	CC	NMI1IC
CAGGGCGACG	UCACACUUGA	CC	NMI1ICR
GGUCAAGUGU	GACGUCGCCC	UG	NMI1R
Group NMI2 :			
GTTCTTGGTC	AAGTGTGACG	TC	NMI2
GACGTCACAC	TTGACCAAGA	AC	NMI2IC
GACGUCACAC	UUGACCAAGA	AC	NMI2ICR
GUUCUUGGUC	AAGUGUGACG	UC	NMI2R
Group NMI3 :			
GCGTTCGTTA	TAGCTATCTA	CTGTGC	NMI3

GCACAGTAGA	TAGCTATAAC	GAACGC	NMI3IC
GCACAGUAGA	UAGCUAUAAC	GAACGC	NMI3ICR
GCGUUCGUUA	UAGCUAUCUA	CUGUGC	NMI3R
Group NMI4 :			
TGCGTTCGAT	ATTGCTATCT	ACTGTGCA	NMI4
TGCACAGTAG	ATAGCAATAT	CGAACGCA	NMI4IC
UGCACAGUAG	AUAGCAAUAU	CGAACGCA	NMI4ICR
UGCGUUCGAU	AUUGCUAUCU	ACUGUGCA	NMI4R
Group NMI5 :			

TTTTGTTCTTGGTCAAGTGTGACGTCGCCCTGAATGGATTCTGTTCCATT NMI5

AATGGAACAGAATCCATTCAGGGCGACGTCACACTTGACCAAGAACAAAA NMI5C

AAUGGAACAGAAUCCAUUCAGGGCGACGUCACACUUGACCAAGAACAAAA NMI5ICR

UUUUGUUCUUGGUCAAGUGUGACGUCGCCCUGAAUGGAUUCUGUUCCAUU NMI5R

Group HDI1 :			
TTATTATGCG	CGAGGCATAT	TG	HDI1
CAATATGCCT	CGCGCATAAT	AA	HDI1IC
CAAUAUGCCU	CGCGCAUAAU	AA	HDI1ICR
UUAUUAUGCG	CGAGGCAUAU	UG	HDI1R
Group BCI1 :			
TTAAACATCT	TACCAAAG		BCI1
CTTTGGTAAG	ATGTTTAA		BCI1IC
CUUUGGUAAG	AUGUUUAA		BCI1ICR
UUAAACAUCU	UACCAAAG		BCI1R
Group BPI1 :			
CCACACCCAT	CCTCTGGACA	GGCTT	BPI1
AAGCCTGTCC	AGAGGATGGG	TGTGG	BPI1IC
AAGCCUGUCC	AGAGGAUGGG	UGUGG	BPI1ICR
CCACACCCAU	CCUCUGGACA	GGCUU	BPI1R

- or a variant sequence which distinguishes of any of the preceding sequences :
  - * either by addition to or removal from any of their respective extremities of one or several nucleotides;
  - * or changing within any of said sequences of one or more nucleotides;
  - * or both;

yet provided that in any of the above circumstances said probe still hybridizes with the same RNA or DNA target as the corresponding unmodified sequence.

4. Probe for detecting one or more Neisseria gonorrhoeae strains, containing :
   - either a sequence belonging to a nucleic acid selected from the following group of nucleic acids and which includes itself of from 15 to the maximum number of nucleotides of the selected nucleic acid :

Group NGI1 :			
CGATGCGTCG	TTATTCTACT	TCGC	NGI1
GCGAAGTAGA	ATAACGACGC	ATCG	NGI1IC
GCGAAGUAGA	AUAACGACGC	AUCG	NGI1ICR
CGAUGCGUCG	UUAUUCUACU	UCGC	NGI1R

   - or a variant sequence which distinguishes of any of the preceding sequences :
     * either by addition to or removal from any of their respective extremities of one or several nucleotides;
     * or changing within any of said sequences of one or more nucleotides;
     * or both;

   yet provided that in any of the above circumstances said probe still hybridizes with the same RNA or DNA target as the corresponding unmodified sequence.

5. Process for detecting Neisseria gonorrhoeae strains in a biological sample, wherein said process comprises contacting said biological sample in which the nucleic acids (DNAs and/or RNAs) of the strains have been made accessible to hybridization, if need be, under suitable denaturation conditions, said nucleic acid to be detected being possibly amplified using the polymerase chain reaction, particularly in which at least one of the probes of claim 4 is used as amplification primer,
with a probe according to anyone of claim 4 under conditions enabling hybridization between the probe and complementary nucleic acids of the Neisseria gonorrhoeae strains, which may be present in the sample, and detecting the hybrids possibly formed, particularly with a probe hybridizing both DNA and RNA of Neisseria gonorrhoeae strain which may be present in the biological sample.

6. Process for detecting Neisseria gonorrhoeae, in a biological sample, according to claim 5, wherein:
the hybridization medium contains about 3 x SSC,
(SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) about 25 mM of phosphate buffer pH 7.1, 20% deionized formamide, 0.02% ficoll, 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, and about 0.1 mg/ml sheared, denatured salmon sperm DNA,
and/or
wash medium contains about 3 x SSC, 25 mM phosphate buffer pH 7.1, and 20% deionized formamide and wherein the probe used is anyone of the probes of claim 4, the hybridization temperature being suitable adjusted to the range of about 50 ° C and/or the wash temperature to the range of about 50 ° C, and particularly wherein said target sequence and the corresponding relevant hybridization temperature (HT) and wash temperature (WT) respectively are as follows:
GCGAAGTAGA ATAACGACGC ATCG
HT and/or WT : 50 ° C.

7. Process for detecting Neisseria gonorrhoeae strains from other organisms, wherein said process comprises contacting said biological sample, in which the nucleic acids (DNAs and/or RNAs) have been made accessible to hybridization, if need be, under suitable denaturation conditions, with two probes, targeting the same nucleic acid molecule, and of which at least one is specific for Neisseria gonorrhoeae and which is selected from any one of the probes of claim 4, whenever required under hybridization and washing conditions adjusted such as to ensure specific hybridization with complementary nucleic acids of the Neisseria gonorrhoeae strains, which may be present in the sample, yet not with complementary DNA or RNA of other organisms and detecting the hybrids possibly formed.

8. Kit for the detection in vitro of a large number, preferably all Neisseria gonorrhoeae strains in a biological sample, said kit containing :
either

- at least one probe selected among any of those according to claim 4;
- the buffer or components necessary for producing the buffer enabling hybridization reaction between these probes and the DNAs and/or RNAs of a large number, preferably all strains of Neisseria gonorrhoeae to be carried out;
- when appropriate means for detecting the hybrids resulting from the preceding hybridization,

or

- at least two probes, targeting the same nucleic acid molecule, and of which at least one is specific for Neisseria gonorrhoeae and which is selected from any one of the probes of claim 4,
- the buffer or components necessary for producing the buffer enabling hybridization reaction between these probes and the DNAs and/or RNAs of a strain of Neisseria gonorrhoeae to be carried out,
- when appropriate means for detecting the hybrids resulting from the preceding hybridization.

9. Probe for detecting one or more Neisseria meningitidis strains, containing :
   - either a sequence belonging to a nucleic acid selected from the following group of nucleic acids and which includes itself of from 15 to the maximum number of nucleotides of the selected nucleic acid :

Group NMI1 :

GGTCAAGTGT	GACGTCGCCC	TG	NMI1
CAGGGCGACG	TCACACTTGA	CC	NMI1IC
CAGGGCGACG	UCACACUUGA	CC	NMI1ICR
GGUCAAGUGU	GACGUCGCCC	UG	NMI1R

Group NMI2 :

GTTCTTGGTC	AAGTGTGACG	TC	NMI2
GACGTCACAC	TTGACCAAGA	AC	NMI2IC
GACGUCACAC	UUGACCAAGA	AC	NMI2ICR
GUUCUUGGUC	AAGUGUGACG	UC	NMI2R

Group NMI3 :

GCGTTCGTTA	TAGCTATCTA	CTGTGC	NMI3
GCACAGTAGA	TAGCTATAAC	GAACGC	NMI3IC
GCACAGUAGA	UAGCUAUAAC	GAACGC	NMI3ICR
GCGUUCGUUA	UAGCUAUCUA	CUGUGC	NMI3R

Group NMI4 :

TGCGTTCGAT	ATTGCTATCT	ACTGTGCA	NMI4
TGCACAGTAG	ATAGCAATAT	CGAACGCA	NMI4IC
UGCACAGUAG	AUAGCAAUAU	CGAACGCA	NMI4ICR
UGCGUUCGAU	AUUGCUAUCU	ACUGUGCA	NMI4R

Group NMI5 :

TTTTGTTCTTGGTCAAGTGTGACGTCGCCCTGAATGGATTCTGTTCCATT NMI5

AATGGAACAGAATCCATTCAGGGCGACGTCACACTTGACCAAGAACAAAA NMI5C

AAUGGAACAGAAUCCAUUCAGGGCGACGUCACACUUGACCAAGAACAAAA NMI5ICR

UUUUGUUCUUGGUCAAGUGUGACGUCGCCCUGAAUGGAUUCUGUUCCAUU NMI5R

- or a variant sequence which distinguishes of any of the preceding sequences :
  - * either by addition to or removal from any of their respective extremities of one or several nucleotides;
  - * or changing within any of said sequences of one or more nucleotides;
  - * or both;

yet provided that in any of the above circumstances said probe still hybridizes with the same RNA or DNA target as the corresponding unmodified sequence.

10. Process for detecting Neisseria meningitidis strains in a biological sample, wherein said process comprises contacting said biological sample in which the nucleic acids (DNAs and/or RNAs) of the

strains have been made accessible to hybridization, if need be, under suitable denaturation conditions, said nucleic acid to be detected being possibly amplified using the polymerase chain reaction, particularly in which at least one of the probes of claim 9 is used as amplification primer,
with a probe according to any one of claim 9 under conditions enabling hybridization between the probe and complementary nucleic acids of the Neisseria meningitidis strains, which may be present in the sample, and detecting the hybrids possibly formed particularly with a probe hybridizing both DNA and RNA of Neisseria meningitidis strain which may be present in the biological sample.

11. Process for detecting Neisseria meningitidis, in a biological sample, according to claim 10, wherein:
the hybridization medium contains about 3 x SSC,
(SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) about 25 mM of phosphate buffer pH 7.1, 20% deionised formamide, 0.02% ficoll, 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, and about 0.1 mg/ml sheared, denatured salmon sperm DNA,
and/or
wash medium contains about 3 x SSC, 25 mM phosphate buffer pH 7.1, and 20% deionized formamide and wherein the probe used is anyone of the probes of claim 10, the hybridization temperature being suitable adjusted to the range of about 40 to 58 °C and/or the wash temperature to the range of about 40 to 58 °C,
and particularly, wherein said target sequence and the corresponding relevant hybridization temperature (HT) and wash temperature (WT) respectively are as follows:

```
CAGGGCGACG  TCACACTTGA  CC
HT and/or WT : 45°C.
GACGTCACAC  TTGACCAAGA  AC
HT and/or WT : 45°C.
GCACAGTAGA  TAGCTATAAC  GAACGC
HT and/or WT : 40°C.
TGCACAGTAG  ATAGCAATAT  CGAACGCA
HT and/or WT : 48°C.
TTTTGTTCTTGGTCAAGGTGTGACGTCGCCCTGAATGGATTCTGTTCCATT
HT and/or WT : 58°C.
```

12. Process for detecting Neisseria meningitidis strains from other organisms, wherein said process comprises contacting said biological sample, in which the nucleic acids (DNAs and/or RNAs) have been made accessible to hybridization, if need be, under suitable denaturation conditions, with two probes, targeting the same nucleic acid molecule, and of which at least one is specific for Neisseria meningitidis and which is selected from any one of the probes of claim 9, whenever required under hybridization and washing conditions adjusted such as to ensure specific hybridization with complementary nucleic acids of the Neisseria meningitidis strains, which may be present in the sample, yet not with complementary DNA or RNA of other organisms and detecting the hybrids possibly formed.

13. Kit for the detection in vitro of a large number, preferably all Neisseria meningitidis strains in a biological sample, said kit containing :
either
- at least one probe selected among any of those according to claim 9;
- the buffer or components necessary for producing the buffer enabling hybridization reaction between these probes and the DNAs and/or RNAs of a large number, preferably all strains of Neisseria meningitidis to be carried out;
- when appropriate means for detecting the hybrids resulting from the preceding hybridization,

or
- at least two probes, targeting the same nucleic acid molecule, and of which at least one is specific for Neisseria meningitidis and which is selected from any one of the probes of claim 9,

- the buffer or components necessary for producing the buffer enabling hybridization reaction between these probes and the DNAs and/or RNAs of a strain of Neisseria meningitidis to be carried out,
- when appropriate means for detecting the hybrids resulting from the preceding hybridization.

14. Probe for detecting one or more Haemophilus ducreyi strains, containing :
    - either a sequence belonging to a nucleic acid selected from the following group of nucleic acids and which includes itself of from 15 to the maximum number of nucleotides of the selected nucleic acid :

Group HDI1 :

TTATTATGCG	CGAGGCATAT	TG	HDI1
CAATATGCCT	CGCGCATAAT	AA	HDI1IC
CAAUAUGCCU	CGCGCAUAAU	AA	HDI1ICR
UUAUUAUGCG	CGAGGCAUAU	UG	HDI1R

    - or a variant sequence which distinguishes of any of the preceding sequences :
        * either by addition to or removal from any of their respective extremities of one or several nucleotides;
        * or changing within any of said sequences of one or more nucleotides;
        * or both;

    yet provided that in any of the above circumstances said probe still hybridizes with the same RNA or DNA target as the corresponding unmodified sequence.

15. Process for detecting Haemophilus ducreyi strains in a biological sample, wherein said process comprises contacting said biological sample in which the nucleic acids (DNAs and/or RNAs) of the strains have been made accessible to hybridization, if need be, under suitable denaturation conditions, said nucleic acid to be detected being possibly amplified using the polymerase chain reaction, particularly in which at least one of the probes of claim 14 is used as amplification primer,
with a probe according to any one of claim 14 under conditions enabling hybridization between the probe and complementary nucleic acids of the Haemophilus ducreyi strains, which may be present in the sample, and detecting the hybrids possibly formed,
particularly with a probe hybridizing both DNA and RNA of Haemophilus ducreyi strain which may be present in the biological sample.

16. Process for detecting Haemophilus ducreyi, in a biological sample, according to anyone of claim 15, wherein :
the hybridization medium contains about 3 x SSC,
(SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) about 25 mM of phosphate buffer pH 7.1, 20% deionized formamide, 0.02% ficoll, 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, and about 0.1 mg/ml sheared, denatured salmon sperm DNA,
and/or
wash medium contains about 3 x SSC, 25 mM phosphate buffer pH 7.1, and 20% deionized formamide and wherein the probe used is anyone of the probes of claim 14, the hybridization temperature being suitable adjusted to the range of about 40 °C and/or the wash temperature to the range of about 40 °C, and particularly, wherein said target sequence and the corresponding relevant hybridization temperature (HT) and wash temperature (WT) respectively are as follows:
CAATATGCCT CGCGCATAAT AA
HT and/or WT : 40 °C.

17. Process for detecting Haemophilus ducreyi strains from other organisms, wherein said process comprises contacting said biological sample, in which the nucleic acids (DNAs and/or RNAs) have been made accessible to hybridization, if need be, under suitable denaturation conditions, with two probes, targeting the same nucleic acid molecule, and of which at least one is specific for Haemophilus ducreyi and which is selected from any one of the probes of claim 22, whenever required under hybridization

and washing conditions adjusted such as to ensure specific hybridization with complementary nucleic acids of the Haemophilus ducreyi strains, which may be present in the sample, yet not with complementary DNA or RNA of other organisms and detecting the hybrids possibly formed.

18. Kit for the detection in vitro of a large number, preferably all Haemophilus ducreyi strains in a biological sample, said kit containing :
either
- at least one probe selected among any of those according to claim 14;
- the buffer or components necessary for producing the buffer enabling hybridization reaction between these probes and the DNAs and/or RNAs of a large number, preferably all strains of Haemophilus ducreyi to be carried out;
- when appropriate means for detecting the hybrids resulting from the preceding hybridization,

or
- at least two probes, targeting the same nucleic acid molecule, and of which at least one is specific for Haemophilus ducreyi and which is selected from any one of the probes of claim 14,
- the buffer or components necessary for producing the buffer enabling hybridization reaction between these probes and the DNAs and/or RNAs of a strain of Haemophilus ducreyi to be carried out,
- when appropriate means for detecting the hybrids resulting from the preceding hybridization.

19. Probe for detecting one or more Branhamella catarrhalis strains, containing :
- either a sequence belonging to a nucleic acid selected from the following group of nucleic acids and which includes itself of from 15 to the maximum number of nucleotides of the selected nucleic acid :

Group BCI1 :

TTAAACATCT	TACCAAAG	BCI1
CTTTGGTAAG	ATGTTTAA	BCI1IC
CUUUGGUAAG	AUGUUUAA	BCI1ICR

UUAAACAUCU	UACCAAAG	BCI1R

- or a variant sequence which distinguishes of any of the preceding sequences :
  * either by addition to or removal from any of their respective extremities of one or several nucleotides;
  * or changing within any of said sequences of one or more nucleotides;
  * or both;

yet provided that in any of the above circumstances said probe still hybridizes with the same RNA or DNA target as the corresponding unmodified sequence.

20. Process for detecting Branhamella catarrhalis strains in a biological sample, wherein said process comprises contacting said biological sample in which the nucleic acids (DNAs and/or RNAs) of the strains have been made accessible to hybridization, if need be, under suitable denaturation conditions, said nucleic acid to be detected being possibly amplified using the polymerase chain reaction, particularly in which at least one of the probes of claim 19 is used as amplification primer, with a probe according to any one of claim 31 under conditions enabling hybridization between the probe and complementary nucleic acids of the Branhamella catarrhalis strains, which may be present in the sample, and detecting the hybrids possibly formed, particularly with a probe hybridizing both DNA and RNA of Branhamella catarrhalis strain which may be present in the biological sample.

21. Process for detecting Branhamella catarrhalis, in a biological sample, according to claim 20, wherein :
the hybridization medium contains about 3 x SSC,
(SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) about 25 mM of phosphate buffer pH 7.1, 20%

deionized formamide, 0.02% ficoll, 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, and about 0.1 mg/ml sheared, denatured salmon sperm DNA,
and/or
wash medium contains about 3 x SSC, 25 mM phosphate buffer pH 7.1, and 20% deionized formamide and wherein the probe used is anyone of the probes of claim 19, the hybridization temperature being suitable adjusted to the range of about 30° C and/or the wash temperature to the range of about 30° C, and particularly, wherein said target sequence and the corresponding relevant hybridization temperature (HT) and wash temperature (WT) respectively are as follows:
CTTTGGTAAG ATGTTTAA
HT and/or WT : 30° C.

22. Process for detecting Branhamella catarrhalis strains from other organisms, wherein said process comprises contacting said biological sample, in which the nucleic acids (DNAs and/or RNAs) have been made accessible to hybridization, if need be, under suitable denaturation conditions, with two probes, targeting the same nucleic acid molecule, and of which at least one is specific for Branhamella catarrhalis and which is selected from any one of the probes of claim 19, whenever required under hybridization and washing conditions adjusted such as to ensure specific hybridization with complementary nucleic acids of the Branhamella catarrhalis strains, which may be present in the sample, yet not with complementary DNA or RNA of other organisms and detecting the hybrids possibly formed.

23. Kit for the detection in vitro of a large number, preferably all Branhamella catarrhalis strains in a biological sample, said kit containing :
either
- at least one probe selected among any of those according to claim 19;
- the buffer or components necessary for producing the buffer enabling hybridization reaction between these probes and the DNAs and/or RNAs of a large number, preferably all strains of Branhamella catarrhalis to be carried out;
- when appropriate means for detecting the hybrids resulting from the preceding hybridization,

or
- at least two probes, targeting the same nucleic acid molecule, and of which at least one is specific for Branhamella catarrhalis and which is selected from any one of the probes of claim 19,
- the buffer or components necessary for producing the buffer enabling hybridization reaction between these probes and the DNAs and/or RNAs of a strain of Branhamella catarrhalis to be carried out,
- when appropriate means for detecting the hybrids resulting from the preceding hybridization.

24. Probe for detecting one or more Bordetella pertussis strains, containing :
- either a sequence belonging to a nucleic acid selected from the following group of nucleic acids and which includes itself of from 15 to the maximum number of nucleotides of the selected nucleic acid :

Group BPI1 :

CCACACCCAT	CCTCTGGACA	GGCTT	BPI1
AAGCCTGTCC	AGAGGATGGG	TGTGG	BPI1IC
AAGCCUGUCC	AGAGGAUGGG	UGUGG	BPI1ICR
CCACACCCAU	CCUCUGGACA	GGCUU	BPI1R

- or a variant sequence which distinguishes of any of the preceding sequences :
  - either by addition to or removal from any of their respective extremities of one or several nucleotides;
  - or changing within any of said sequences of one or more nucleotides;
  - or both;

yet provided that in any of the above circumstances said probe still hybridizes with the same RNA or DNA target as the corresponding unmodified sequence.

25. Process for detecting Bordetella pertussis strains in a biological sample, wherein said process comprises contacting said biological sample in which the nucleic acids (DNAs and/or RNAs) of the strains have been made accessible to hybridization, if need be, under suitable denaturation conditions, said nucleic acid to be detected being possibly amplified using the polymerase chain reaction, particularly in which at least one of the probes of claim 24 is used as amplification primer,
with a probe according to any one of claim 24 under conditions enabling hybridization between the probe and complementary nucleic acids of the Bordetella pertussis strains, which may be present in the sample, and detecting the hybrids possibly formed, particularly with a probe hybridizing both DNA and RNA of Bordetella pertussis strain which may be present in the biological sample.

26. Process for detecting Bordetella pertussis, in a biological sample, according to claim 24, wherein:
the hybridization medium contains about 3 x SSC,
(SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) about 25 mM of phosphate buffer pH 7.1, 20% deionized formamide, 0.02% ficoll, 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, and about 0.1 mg/ml sheared, denatured salmon sperm DNA,
and/or
wash medium contains about 3 x SSC, 25 mM phosphate buffer pH 7.1, and 20% deionized formamide and wherein the probe used is anyone of the probes of claim 40, the hybridization temperature being suitable adjusted to the range of about 55 ° C and/or the wash temperature to the range of about 55 ° C, and particularly, wherein said target sequence and the corresponding relevant hybridization temperature (HT) and wash temperature (WT) respectively are as follows:
AAGCCTGTCC AGAGGATGGG TGTGG
HT and/or WT : 55 ° C.

27. Process for detecting Bordetella pertussis strains from other organisms, wherein said process comprises contacting said biological sample, in which the nucleic acids (DNAs and/or RNAs) have been made accessible to hybridization, if need be, under suitable denaturation conditions, with two probes, targeting the same nucleic acid molecule, and of which at least one is specific for Bordetella pertussis and which is selected from any one of the probes of claim 24, whenever required under hybridization and washing conditions adjusted such as to ensure specific hybridization with complementary nucleic acids of the Bordetella pertussis strains, which may be present in the sample, yet not with complementary DNA or RNA of other organisms and detecting the hybrids possibly formed.

28. Kit for the detection in vitro of a large number, preferably all Bordatella pertussis strains in a biological sample, said kit containing :
either
- at least one probe selected among any of those according to claim 24;
- the buffer or components necessary for producing the buffer enabling hybridization reaction between these probes and the DNAs and/or RNAs of a large number, preferably all strains of Bordetella pertussis to be carried out;
- when appropriate means for detecting the hybrids resulting from the preceding hybridization,

or
- at least two probes, targeting the same nucleic acid molecule, and of which at least one is specific for Bordetella pertussis and which is selected from any one of the probes of claim 24,
- the buffer or components necessary for producing the buffer enabling hybridization reaction between these probes and the DNAs and/or RNAs of a strain of Bordetella pertussis to be carried out,
- when appropriate means for detecting the hybrids resulting from the preceding hybridization.

Fig. 1


AGAGAAAGAAGGGGCTTTAGGCATTCACACTTATCGGTAAACTGAAAAGA -50
AGAGAAAGAAGGGGCTTTAGGCATTCACACTTATCGGTAAACTGAAAAGA -50
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CCACCAAAACTTTACAAATGAAAGCAAGTTTGCTGTTTTTAGCAGCTTAT -300
CCACCAAAACTTTACAAATGAAAGCAAGTTTGCTGTTTTTAGCAGCTTAT -300
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TTTGATTTGCGAAGTAGAATAACGACGCATCGATC

Fig. 2

```
AAGAGCTTGAGTGCTCGTGTCAAGTGTCCACGCTTATCGGTTGTTGTTAT -50
::::::::::::::::::::::::::::::::::::::::::::::::::
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ATAGCTGCTGGATCGGTGGCTGCTGATCCGAGAGAGAAAGGTTTCGCGGG -100
::::::::::::::::::::::::::::::::::::::::::::::::::
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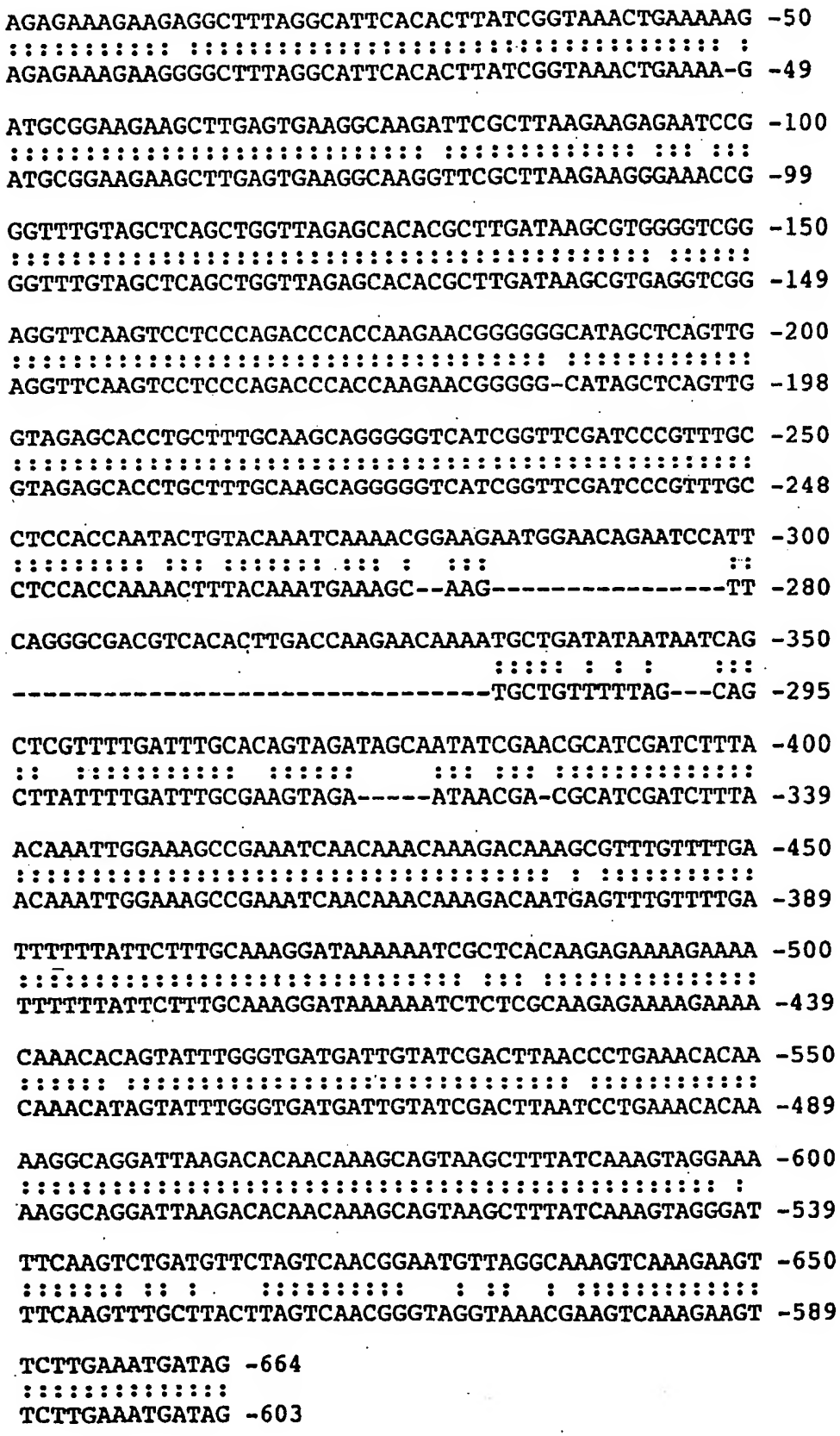
Fig. 3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TTCAAGTTTGCTTACTTAGTCAACGGGTAGGTAAACGAAGTCAAAGAAGT -589
TCTTGAAATGATAG -664
TCTTGAAATGATAG -603

Fig. 4

```
A-GAGAAAGAAGGGGCTTT--AGGCATTCACACTTATCGGTAAACTGAAA -47
: :::   ::  :  : :   : :  : ::: :::::::::    ::
AAGAGCTTGAGTGCTCGTGTCAAGTGTCCACGCTTATCGGT----TGTTG -46

AGATGCGGAAGAAGCTTGAGTGAAGGCAAGGTTCGCTTAAGAAGGGAAAC -97
  ::   :  :  :  :  :::   ::  : : ::    :::: ::   :
TTATATAGCTGCTGGATCGGTGGCTGCT-GATCCGAGAGAGAAAGGTTTC -95

-CGGGTTTGTAGCTCAGCTGGTTAGAGCACACGCTTGATAAGCGTGAGGT -146
 ::::: ::::::::::  ::::::::::   :::::::::   : :::
GCGGGTCTGTAGCTCAGTCGGTTAGAGCACCGTCTTGATAAGGCGGGGGT -145

CGGAGGTTCAAGTCCTCCCAGACCCACCAAG---------------AACG -181
::  ::::: : :::  ::::::::::::::                  :: :
CGTTGGTTCGAATCCAACCAGACCCACCAAGGTTTCCTGAGAGGGAAATG -195

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::::  :::::::: ::: ::::: :::::::::::::::::  ::::::
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:::::::::::::  :::::::::::: : :  : :  : : :  ::
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GCTGTTTTTAGCAGCTTATTTTGATTTGCGAAGTAGAATAACGACGCATC -331
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AAGTAGGGATTTCAAGTTTGCTTACTTAGTCAA-CGGGTAGGTAAACGAA -578
 : :   : ::: :: ::   : :       ::: :: : : : : :  :
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GTCAAAGAAGTTCTTGAAATGATAG -603
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Fig. 5

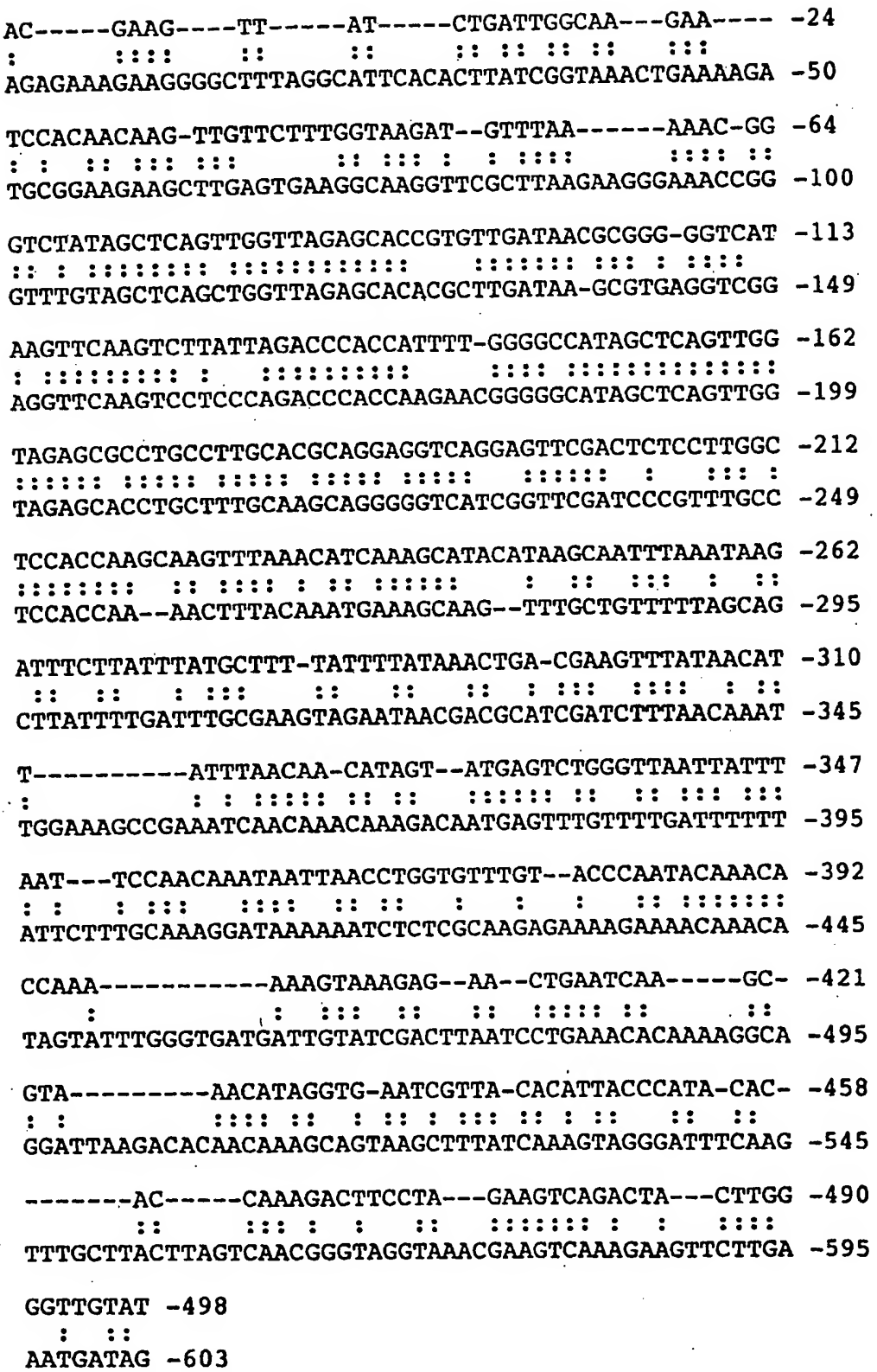
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Fig. 6

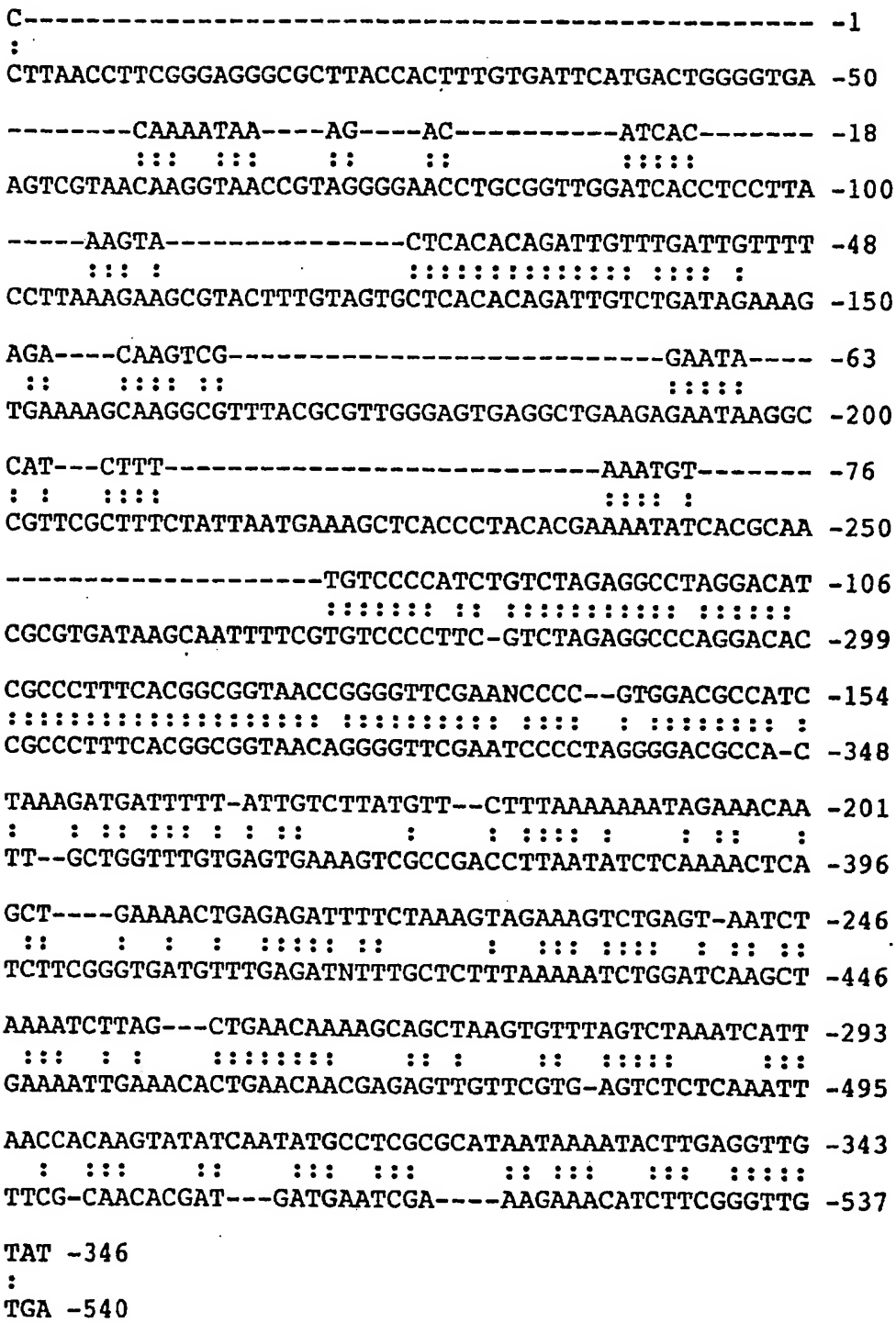
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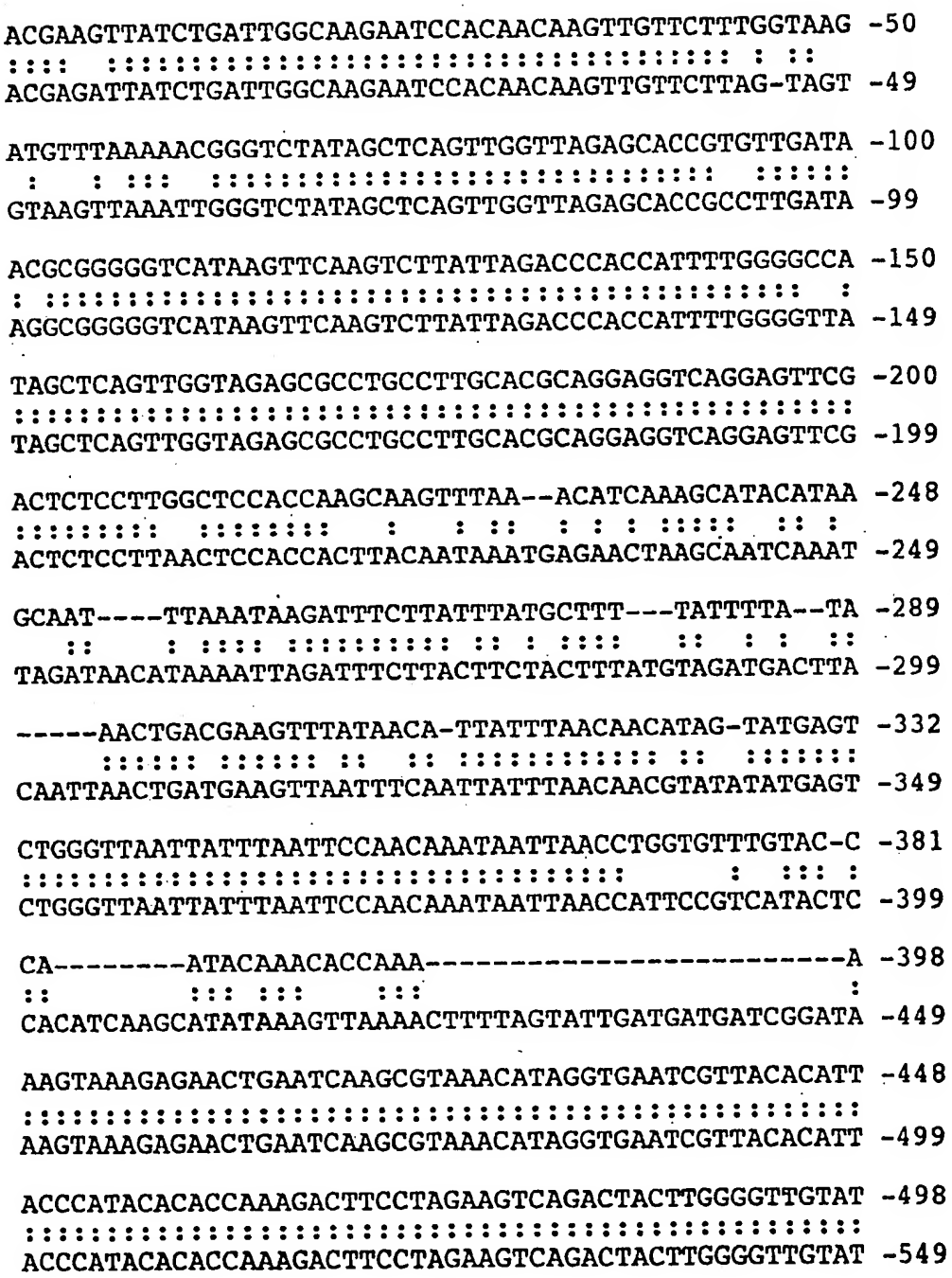
Fig. 7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EUROPEAN SEARCH REPORT

Application Number

EP 90 40 1054

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	CHEMICAL ABSTRACTS, vol. 112, March 1990, page 224, abstract no. 93063s, Columbus, Ohio, US; R.A. TORRES et al.: "Species and genus specificity of the intergenic spacer (IGS) in the ribosomal RNA genes of Cucurbitaceae", & BIOSCI 1989, 44(11-12) 1029-34 * Abstract *	1	C 12 Q 1/68 C 07 H 21/04
A	CHEMICAL ABSTRACTS, vol. 112, May 1990, page 217, abstract no. 173511b, Columbus, Ohio, US; G. HIDE et al.: "The identification of Trypanosoma brucei subspecies using repetitive DNA sequences", & MOL. BIOCHEM. PARASITOL. 1990, 39(2), 213-25 * Abstract *	1	
A	CHEMICAL ABSTRACTS, vol. 109, September 1988, page 391, abstract no. 89552w, Columbus, Ohio, US; W.B. EVANS et al.: "The use of DNA probes for taxonomic study of Dictyostelium wild isolates", & GENETICS 1988, 119(3), 561-9 * Abstract *	1	
A	BIOLOGICAL ABSTRACTS, vol. 83, November 1987, abstract no. 104704, Philadelphia, PA, US; M. VERMA et al.: "Phylogenetic implication of heterogenecity of the nontranscribed spacer of ribosomal DNA repeating melt in various Neurospore and related fungal species", & CURR. GENET. 11(4): 309-314. 1987 * Abstract *	1	TECHNICAL FIELDS SEARCHED (Int. Cl.5) C 12 Q
A	EP-A-0 307 270 (INSTITUT PASTEUR) * Abstract; pages 3,4; claims 1-5 *	1	

-/-

The present search report has been drawn up for all claims

Place of search	Date of completion of search	Examiner
The Hague	16 December 90	OSBORNE H.H.

CATEGORY OF CITED DOCUMENTS

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T: theory or principle underlying the invention

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D: document cited in the application
L: document cited for other reasons

&: member of the same patent family, corresponding document

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 40 1054

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
P,A	EP-A-0 395 292 (BIORESEARCH IRELAND) * Abstract; page 6, lines 40-41 *	1	
A	EP-A-0 337 896 (N.V. INNOGENETICS S.A)		
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)

The present search report has been drawn up for all claims

Place of search	Date of completion of search	Examiner
The Hague	16 December 90	OSBORNE H.H.

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Y: particularly relevant if combined with another document of the same catagory
A: technological background
O: non-written disclosure
P: intermediate document
T: theory or principle underlying the invention

E: earlier patent document, but published on, or after the filing date
D: document cited in the application
L: document cited for other reasons

&: member of the same patent family, corresponding document